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Recent Advances in Electron-Transfer Reactions

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1 INTRODUCTION

Although for a number of years interest has been shown in the theoretical aspects of electron transfer[1-6], it is only very recently that experimentalists have investigated systems where some clear-cut interpretation of results is possible. Several reviews covering both theory of and experiments on electron-transfer processes have been published[7-10]; in this review an attempt is made to summarize the more important results of the last two years or so, especially the results obtained in the chemistry of bridged complexes by Taube and his co-workers at Chicago.

Electron transfer is very often invoked as a step in an oxidation-reduction process: for example, in the reaction $Fe^{3+} + Cr^{2+} \rightarrow Fe^{2+} + Cr^{3+}$, the obvious net change is the loss of an electron by the chromous ion and the gain of one electron by the ferric ion. The questions which immediately arise are (i) whether the electron lost by the chromium and gained by the iron is the same, and (ii) if only the one electron is involved, how does it move from chromium to iron?

Conferences held since 1952 have resulted in a fairly clear delineation of the forms of the activated complex in electron-transfer reactions and two experimentally important forms have been advanced. One is the bridged activated complex — that is the complex in which at least one atom or ligand is common to the coordination spheres of both reactant ions; if the two ions react through this activated complex, there must be changes in the first coordination sphere of at least one of the ions. The second form for the activated complex is the outer sphere type, in which no interpenetration of the first coordination spheres of the ions takes place, and so both the number and the nature of substituent groups remains unaltered on electron transfer. The possibility also exists that the electron may be transferred first to the solvent, then later from the solvent to the other reactant. While this may take place in liquid ammonia, it is not likely to do so in water, except with the most powerful reducing agents — possibly the observations of the decomposition of water by $[Co(CN)_6]^{4-}$ may be explained this way[11].

1.1 *Factors influencing the rate of electron transfer*

In general, factors which will influence the rate of electron transfer are size, electron distribution and structure of the reacting ions, the free energy change and the rearrangement requirements of both the solvent atmosphere and of the substituent groups. The smaller the radii of the reacting ions, the greater the coulombic repulsion in the activated complex at distances suitable for electron transfer. This repulsion can be decreased by the introduction of an ion of opposite charge between the two reacting ions giving rise to bridged complexes. To

be more specific, let us for a moment consider the effect of added anions on the rate of electron transfer between two cations. The acceleration may be due either to the formation of bridged complexes with the electron transfer taking place through the ligand, or to the formation of ion pairs with one of the reacting ions, thereby making other paths available for the electron-transfer process. Acceleration of the process may also be caused by ligands complexed in the first coordination shell of the reacting ion but which are not in a bridging position.

The Franck-Condon principle imposes some restrictions on the electron-transfer process. Since the nuclei may be assumed stationary during the actual electron transfer, the energies of the electronic states of the two reacting ions must be made equal before the transfer can occur. The overall process consists of the approach of the two ions, the formation of an activated complex, the rearrangement of the electronic structure of the complex so that when the electron is transferred energy is conserved, and the final rearrangement of the activated complex and dissociation into products. Some confusion has existed over the interpretation of the Franck-Condon limitations, for earlier it was believed[2] that an electron was transmitted more rapidly than the substituent atmospheres could rearrange, resulting in reduced ions surrounded by their unreduced environment and oxidized ions in the reduced environments. The later rearrangement of the atmospheres required energy and this constituted a barrier to the electron-transfer process. Subsequent modifications[10] have introduced the concept of energy matching at the two sites, this process sometimes requiring considerable energy. Thus although the actual electron transfer is rapid, the rate of reaction may be much slower, due to the low probability of barrier penetration (transmission coefficient $K_e << 1$). The energy required to move the electron must be supplied by the energy of binding in its final position. For a reaction involving no net chemical change, the activated complex must be symmetrical when the electron is half-way transferred and at this point the metal centres should be indistinguishable to the electron.

The energy of activation may be expressed as, $\Delta F^{\ddagger}_{app} = -RT \ln K_2 + \Delta F^{\ddagger}_{rep} + \Delta F^{\ddagger}_{r}$ (where $\Delta F^{\ddagger}_{rep}$ is the coulombic repulsion between the reacting centres and $\Delta F^{\ddagger}_{r}$ the energy required for rearrangement of the ion environment). For outer sphere complexes where the solvent rearrangements take place beyond the first coordination shell of the ions and require only small rearrangement energy, the rate of electron transfer should be faster than in bridged complexes. However, the actual rate will also be modified if the radius of the first coordination sphere varies greatly with valency change (e.g., $[Co(NH_3)_6]^{3+}$, $[Co(NH_3)_6]^{2+}$), slightly (e.g., $[Fe(H_2O)_6]^{3+}$, $[Fe(H_2O)_6]^{2+}$) or not at all (e.g., MnO_4^{2-}, MnO_4^{-})[4, 10]. This trend may be altered, and even reversed, if the crystal-field strength of the ligand causes a change in spin multiplicity of the metal, or if the ligand possesses electron-conducting or insulating properties. It is found that electron transfer between Co(II) and Co(III) proceeds more rapidly when the surrounding ligands are water (low crystal-field strength) or ethylenediamine (high crystal-field strength) than with EDTA or ammonia (medium crystal-field strength). This is because in the limits of zero crystal-field energy both Co(II) and Co(III) become spin-free; at high crystal fields they become spin-paired. When the ligand is *o*-phenanthroline, the rate is very much greater than would be expected from crystal-field strength alone; apparently conjugated π-systems can conduct the electron from the metal to the periphery of the complex ion, making the exchange process more rapid. Dipyridyl (2,2′dipyridine) behaves similarly.

Oxidation-reduction reactions will be slower between one-electron oxidants and two-electron reductants (or vice versa) than between one-electron oxidants and one-electron reductants, since reaction between the former must proceed either through termolecular paths or by the formation of unstable intermediates[10]. However, if the entropy of activation is particularly favourable, or if intermediate oxidation states (such as divalent thallium) exist, then the reaction rate can still be appreciable[13].

2 THE BRIDGED ACTIVATED COMPLEX

In the electron-transfer process which takes place through a bridged activated complex,

two possibilities arise; the bridging group may be transferred from one ion to the other or it may remain associated with one ion. If during the electron transfer a negatively charged group moves from the oxidant to the reductant, the process becomes equivalent to an atom transfer, which should require less reorganization of the ion atmospheres and may thus provide a more favourable path for reaction. Mechanisms have also been proposed involving the shift of a proton from the shell of the reductant to that of the oxidant during electron transfer. It has been found, for example, that electron transfer between Fe(II) and Fe(III) is accelerated by sulphuric acid, the proposed mechanism being[14],

$$[(H_2O)_5Fe^{II}OSO_3H\ .]^+ \ldots . \; [.\ O_3SOFe^{III}(H_2O)_5]^+ \rightleftharpoons [(H_2O)_5Fe^{III}OSO_3\ .]^+ \ldots . \; [.\ HO_3SOFe^{II}(H_2O)_5]^+$$

Much has been made of this theory of hydrogen atom transfer for the oxidation-reduction process[15–17] and although possibly correct, there is no definite proof that it occurs.

The simplest demonstrations of group transfer have come from studies of Co(III) and Cr(III) complexes, and Cr(II) in aqueous solution. Since if a negative group is to be transferred from oxidant to reductant, one metal-ligand bond must be broken and one metal-ligand bond formed, the process will be easiest when the oxidant is substitution-inert before reaction and substitution-labile after electron transfer (i.e. in the reduced state), and when the reductant is labile before reaction and proceeds to an inert oxidation state after transfer. These requirements are satisfied by the above complexes and definite proof for electron transfer through bridging groups has been obtained only in the reactions of Co(III) and Cr(III) with Cr(II) or Co(II), of Pt(II) with Pt(IV) complexes[18], and more recently of Co(III) with V(II) complexes[19].

We might consider, as an example, one of the first demonstrations of electron transfer through a bridged complex[20], the reaction of $[(NH_3)_5CoCl]^{2+}$ with Cr^{2+} to give NH_4^+, Co^{2+} and $CrCl^{2+}$. Since Cr(III) substitutes very slowly, the Cr-Cl bond must have been formed in the activated complex; it could not have formed between Cr^{3+} and Cl^- since when radioactive Cl^- is added to the solution no radioactivity is found in the $CrCl^{2+}$. The oxidant $[(NH_3)_5CoCl]^{2+}$ is substitution inert, hence the Co-Cl bond must exist in the activated complex.

Similar methods have shown that electron transfer by bridging occurs with ligands such as the halides, CNS^-, N_3^-, SO_4^{2-}, PO_4^{3-}, $P_2O_7^{4-}$, carboxylic acids, and water (H_2O and OH^-). The rates found for the reaction of some Cr(III) complexes with Cr^{2+} are shown in Table 1. In general, specific rate constants are very low, but increase along the series $F < Cl < Br < I$, supporting the view that electron transfer takes place through the ligand rather than directly to the *d*-orbitals of the oxidant.

The rate of reaction of the hexammino-cobalt(III) ion, $[(NH_3)_6Co]^{3+}$, is 100 times slower than that of the pentamminoaquo-cobalt(III) ion when the reductant is substitution labile, and this difference is apparently due to the availability of a pair of unshared electrons in a coordinated water molecule. When $Cr^{2+}_{aq.}$ is the reductant, it has now been conclusively demonstrated that the electron transfer takes place through a bridging $-OH_2$ or $-OH$ group[21, 22]. The mechanism for reaction of the hexammine ion is not known with certainty, and recently it has again been suggested[23] that the reaction takes place by dissociation of an ammonia molecule to give a proton,

$$[(NH_3)_6Co]^{3+} \rightleftharpoons [(NH_3)_5CoNH_2]^{2+} + H^+.$$

Thus, in the isotopic redox process,

$$[(NH_3)_6Co]^{3+} + [(NH_3)_6Co]^{2+} \rightarrow [(NH_3)_6Co]^{2+} + [(NH_3)_6Co]^{3+},$$

the reaction is assumed to involve the production of a proton from the Co(III) ion, followed by electron transfer. The specific rate constant found is given by $\log k = 4.42 - 13.5/2.303\,RT$ ($= 3.4 \times 10^{-6}$ 1. mole^{-1} sec^{-1} at 25°C) and agrees well with a previous value of $< 7 \times 10^{-6}$. The new work shows that introduction of $-OH_2$ in place of $-NH_3$ increases the rate by a factor of 20, although ΔE remains constant at 13.5 kcal mole^{-1}. The mechanism proposed presumably requires the formation of a bridged activated complex.

Taube, on the other hand, tends to favour an outer sphere mechanism and his opinion is based on the following evidence: (i) the rate of reaction of $[(NH_3)_6Co]^{3+}$ is smaller than that of $[(NH_3)_5CoOH_2]^{3+}$ with $Cr^{2+}_{aq.}$, (ii) when an outer sphere reductant such as

Table 1 Specific rate constants for reactions between Cr^{2+} and some Cr(III) complexes[7]

Complex	k (l. mole^{-1}sec^{-1})	T (°C)
CrF^{2+}	2.6×10^{-2}	27
$CrCl^{2+}$	9 ± 1	0
$CrBr^{2+}$	60	0
$[(NH_3)_5CrF]^{2+}$	2.7×10^{-4}	25
$[(NH_3)_5CrCl]^{2+}$	5.1×10^{-2}	25
$[(NH_3)_5CrBr]^{2+}$	3.2×10^{-1}	25
$[(NH_3)_5CrI]^{2+}$	5.5 ± 1.5	25
CrN_3^{2+}	1.2	0
$CrNCS^{2+}$	1.8×10^{-4}	27
trans-$CrCl_2^+$	23×10^2	—

$[Cr(dipy)_3]^{2+}$ is used, the specific rate constant of the reaction of the hexammine cobalt(III) ion increases by a factor of 10^6 — i.e., from 3×10^{-4} to $> 7 \times 10^2$ l. mole^{-1} sec.$^{-1}$ at 25°C.[24] The available energy does not appear to be enough to supply the high activation energy needed for dissociation and this difficulty is avoided by postulating reaction through an outer sphere complex. However, the reaction of the pentamminoaquocobalt(III) ion with $[Cr(dipy)_3]^{2+}$ yields some surprising results which must be considered. Thus the rate of reaction of $[(NH_3)_5CoOH_2]^{3+}$ is greater than that of $[(NH_3)_6Co]^{3+}$ with $Cr^{2+}_{aq.}$ by a factor of 100; with $[Cr(dipy)_3]^{2+}$, the rate is still greater than that of the hexammino complex, by a factor of 90. Since the pentamminoaquo complex is known to react by bridging with $Cr^{2+}_{aq.}$, the mechanism for the hexammino complex still appears to be in doubt.

Because of the very low rate of electron transfer across the ammonia ligand, this group may be used to block electron transfer to a central Co(III) or Cr(III) in five of the six coordination positions, allowing electron transfer only through a bridging group in the sixth position. The point of attack at the ligand by the reductant is of some interest. Ball and King demonstrated[25] by the use of NCS^- and N_3^- as bridging groups that the rate of reaction with Cr^{2+} is greater (Table 1) when a stable Cr(III) complex can be formed by attack at the end of the ligand remote from the Cr(III) centre (possible for N_3^- but not NCS^-). Unfortunately, there are very few inorganic ligands where such a test is possible and in more recent work carboxylic acids have been used as bridging groups. This continues to be a most promising field of study, since a great number of subtle variations may be built into the ligand under study. In all systems so far examined, the basic oxidant group is the pentamminocobalt(III) ion with the sixth position filled by the organic acid. For complexes such as the acetato $[(NH_3)_5Co{-}O{-}CO{-}CH_3]^{2+}$ or butyrato, attack by the chromous ion can take place only at the carbonyl group as is shown by the very close similarity of the rates of reaction for the acetato, succinato, and methylsuccinato derivatives shown in Table 2.

Table 2 Specific rate constants of reaction[7] *of Cr^{2+} with $[(NH_3)_5CoX]^{2+}$*

Ligand X	k (l. mole^{-1}sec^{-1})	T (°C)
O_2CCH_3	0.18	25.1
$O_2CCH_2CH_2CO_2H$	0.17	14.1
$O_2CCH_2CH_2CO_2CH_3$	0.17	25.0
O_2CCH_2Cl*	0.11	29.5
O_2CCHCl_2*	0.08	30.5
O_2CCF_3*	0.07	30.0

* Fraser, unpublished results

As might be expected, the rate of electron transfer between the Co(III) complex and Cr^{2+} decreases along the series $-O_2CCH_3$, $-O_2CCH_2Cl$, $-O_2CCHCl_2$, $-O_2CCCl_3$ as the number of electronegative groups is increased[26].

Taube and Sebera[7] found that with fumaric acid as ligand, the rate was not similar to that for the succinato complex but was much greater and showed a first-order dependence on the pH of the solution. To account for this, they postulated that attack by chromous ion takes place at the carbonyl remote from the cobalt centre, with subsequent electron transfer through the organic ligand. The acceleration by acid was attributed to better conjugation between the metal centres (Figure 1).

They observed this acceleration by acid not only with the fumarato complex, but also with the methyl and the *p*-phthalato esters of fumaric acid, but not with the *o*- or *m*-

phthalato complexes where conjugation between the metal centres is impossible.

Support for the theory of remote attack has been obtained from two sources:

(1) When the methylsuccinato complex reacts with Cr^{2+}, it can be shown by titration that no hydrolysis of the methyl succinate occurs. In all cases the reaction may be represented by the equation

$$[(NH_3)_5CoX]^{2+} + 5H^+ + Cr^{2+} \rightarrow 5NH_4^+ + Co^{2+} + CrX^{2+}$$

and the amount of free acid is easily calculated. When the complex is methylfumarato, however, titration shows the increase of one equivalent in H^+ content after reaction, and ether extractions show that both fumaric acid and methyl alcohol are associated with the chromium[27]. It has been proved that the alcohol does not go into solution to become associated with the chromium and similar results have been obtained with phenyl fumarate and with methyl terephthalate as ligands.

(2) The ability of an electron to be transferred through a conjugated ligand has been demonstrated in the oxidation of $[(NH_3)_5CoO_2CCO_2]^+$ and $[NH_3)_5CoO_2CC_6H_4CHO]^{2+}$ with a series of oxidants[28, 29]. With two-electron oxidizing agents such as chlorine, the valency state of the cobalt is preserved, and the products are $[(NH_3)_5CoH_2O]^{3+}$ and $[(NH_3)_5CoO_2CC_6H_4CO_2H]^{2+}$; with one-electron oxidizing agents, however, the cobalt is reduced to Co^{2+}. Apparently one electron moves to the oxidant (Ag^+, $S_2O_8^{2-}$:Co^{3+}) while the other moves through the system to the Co(III) centre.

With a dibasic ligand bound at only one point, the reductant may attack at a number of different places on the ligand. For example, the fumarato complex and chromous ion may react (i) by remote attack, non-protonated path, (ii) remote attack, protonated path, (iii) remote attack, anion path and (iv) adjacent attack, depending on the acid content of the solution. These situations are illustrated in Figure 2.

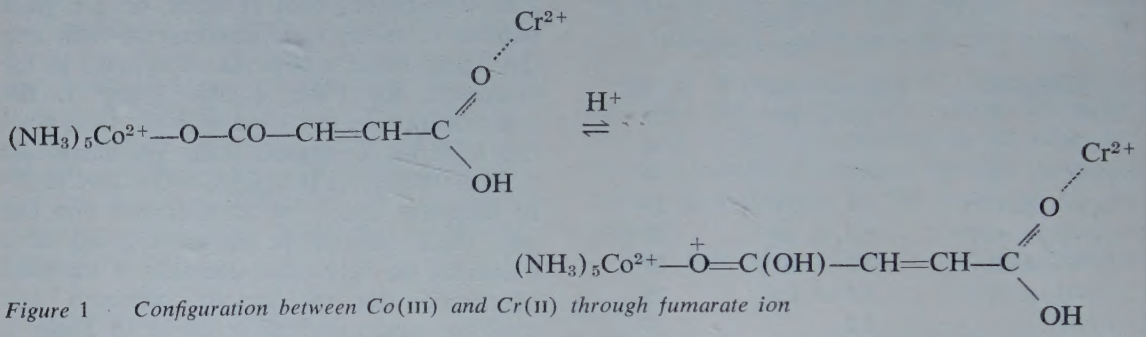

Figure 1 *Configuration between Co(III) and Cr(II) through fumarate ion*

Evidence for reaction by path (iii) has been found by Sebera and Taube[30]. When the ligand is an ester, the possibility of path (iii) no longer exists. A recent study[31] has shown that with cyclohexylterephthalato, ethylterephthalato, methylfumarato and phenylfumarato complexes, the reaction with Cr^{2+} takes place by paths (i), (ii) and (iv) simultaneously, whereas with the phenylterephthalato and *p*-cresylterephthalato complexes, reaction by path (i) either vanishes or takes place very slowly. This difference is also shown in the form of the rate laws for the complexes[32]: for methyl fumarato, rate = 0.42 (Cr^{2+}) (CoL^{2+}) + 1.1 (H^+) (Cr^{2+}) (CoL^{2+}) at 5°C[30], for phenyl fumarate, rate = 1.38 (Cr^{2+}) (CoL^{2+}) + 0.61 (Cr^{2+}) (CoL^{2+}) (H^+) at 22°C but for phenyl and cresyl terephthalate[31], the rates are respectively equal to 0.05 (Cr^{2+}) (CoL^{2+}) + 0.4 (Cr^{2+}) (CoL^{2+}) (H^+) at 24°C and 0.04 (Cr^{2+}) (CoL^{2+}) + 0.19 (Cr^{2+}) (CoL^{2+}) (H^+) at 26°C (rate for *o*-phthalato = 0.05 (Cr^{2+}) (CoL^{2+}) at 14°C)[7]. Although the theory of electron transport by bridging groups is not as well understood as that for outer sphere complexes, a recent paper by Orgel and Halpern[33] has pointed out that the rate of electron transfer through a conjugated organic ligand should be proportional to the bond order of the system. They suggest that

other things being equal, electron transfer between t_{2g} *d*-orbitals will be favoured by π-bridging and between e_g orbitals by σ-bridging.

Among the factors which will control the overall rate of the reaction are the need for the ligand to adopt a favourable configuration for the transfer, the rearrangement of electron atmosphere required and the ease of substitution of the reductant at the point of attack to give sufficient overlap of orbitals to allow transfer of the electron. A start has been made to examine some of these effects[32]. Thus, the rate of reaction has been measured by either keeping a terephthalate or fumarate chain and varying the nature of the remote functional group or by keeping the carboxyls and varying the nature of the conducting system. Some results are shown in Table 3, and it may be seen that whilst $-COOH$, $-COOR$, $-CHO$, $-CRO$, $-CONH_2$ and $-CONHCH_3$ are groups which allow remote attack, $-SO_2OH$, $-OH$, $-CN$ and $-CON(C_2H_5)_2$ do not. Neither *cis-* nor *trans-cyclo*propane rings act as conductors for the electron transfer process. It is at present difficult to explain the ester hydrolysis and the results for the amide, since a formal scheme for the electron transfer process may be written which does not involve the alkoxy group[30] (Figure 3).

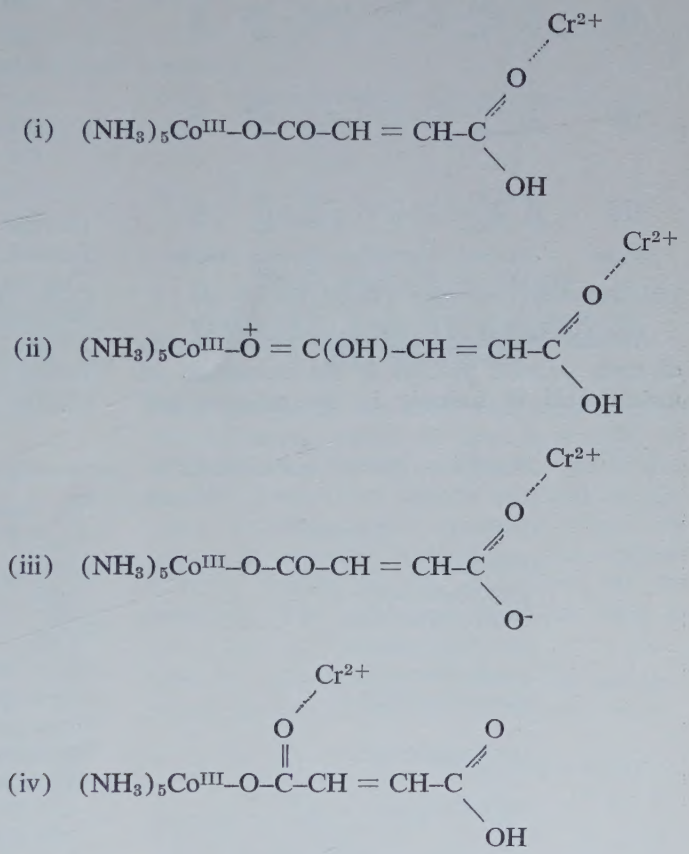

Figure 2 Possible paths for reaction between Cr^{2+} and $[(NH_3)_5Co(O_2CCHCHCO_2H)]^{2+}$

On the other hand, after reaction, the remote carboxyl will be doubly substituted

$$[CH_3O–\underset{|}{\overset{|}{C}}–OCr(III)]$$

and because of the inert nature of the Cr(III), breaking of the C–O–CH_3 system rather than the C–O–Cr(III) is not very surprising. This argument cannot be applied to cases when V^{2+} or Eu^{2+} are the reductants, even though similar results have been obtained. Apparently the process involves rearrangement of the electronic distribution and abstraction of the electrons from the O–CH_3 bond. Experiments using ^{18}O enriched water show that alkyl-oxygen fission occurs to 97% with the methyl

esters and up to 15% with the phenyl ester[34]; alkyl-oxygen fission can also be demonstrated using optically active or crotyl esters[35].

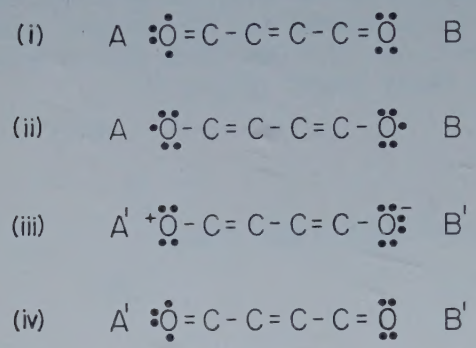

Figure 3 *Formal scheme for electron transfer according to Sebera and Taube*[30]

Another interesting activation caused by the electron transfer process is the isomerism of maleic acid to fumaric in the maleato and methyl maleato complexes[36]. It is found that the ratio of fumaric to maleic acids increases linearly with increasing hydrogen ion concentration of the solutions. Table 4 shows the ratios obtained using the two complexes; with the acid complex the *cis*-configuration is retained at higher acid concentration. This difference may indicate that possibly chelation in the activated complex is easier with the maleato than methyl maleato, thus preserving the configuration[32]. The observation that carbon-deuterium bonds are formed with these two complexes when D_2O is used as solvent, but not in the electron transfer reaction through fumarate, suggests that a radical is formed when maleate is the bridging ligand. This suggestion has some theoretical support[33], and experimentally it is found that the electron transfer through maleate is fast, whereas through *o*-phthalate (with almost the same relative configuration) it is slow, which points to a different mechanism in the two cases. It should be mentioned that the reaction of the crotonato complex with Cr^{2+} is slow, whereas with $[Cr(dipyridyl)_3]^{2+}$ it is very fast, which suggests that the electron can be added from the outer sphere Cr(II) complex directly to the conjugated system.

Table 4 *Ratio of fumaric to maleic acid produced on electron transfer*

$[H^+]$ (*M*)	maleato complex	methyl maleato
0.045	0.05	0.11
0.2	0.13	1.84
0.3	0.26	3.0
0.5	0.47	5.2

Table 3 *Specific rate constants for pentamminocobalt(III) complexes and chromous ion*

Complex	k_{obs} (l. mole^{-1}sec^{-1})	T (°C)
benzoato*	0.16	27
p-sulphobenzoato	0.16	27
p-aldehydobenzoato	~500	20
p-hydroxybenzoato*	0.14	26
p-cyanobenzoato*	0.18	26
phenylterephthalato	0.13 ($H^+ = 0.2\,M$)	24
	0.29 ($H^+ = 0.6\,M$)	24
cresylterephthalato*	0.078 ($H^+ = 0.2\,M$)	26
	0.152 ($H^+ = 0.6\,M$)	26
phenylfumarato	1.45 ($H^+ = 0.1\,M$)	22
	1.60 ($H^+ = 0.4\,M$)	22
cis-cyclopropanedicarboxylato	0.22	30.5
trans-cyclopropanedicarboxylato	0.15	25
amidofumarato	0.35	25
diethylamidofumarato	0.15	25

* Fraser, unpublished results

Kopple and Svartos[37] using pentamminocobalt(III) complexes have studied electron transfer through a protein molecule. The cobalt(III) complexes were prepared contain-

ing water soluble copolymers of DL-alanine and L-glutamic acid and it was found that the reaction with chromous ion proceeds at two rates, differing by a factor of 400 (Table 5). It was postulated that the high reaction rate corresponds to electron transfer through the ligand utilizing a path of carboxyl groups overlapping a system of hydrogen-bonded amide groups. The slow rate of reaction presumably characterizes attack by the chromous ion at carboxyls adjacent to a substituted cobalt(III) centre. Investigations of this system are continuing.

Table 5 Specific rate constant for copolymer complexes[37]

Copolymer	T (°C)	k (l. mole^{-1}sec^{-1})	Conversion (%)
DL-alanine (84%) + L-glutamic acid (16%)	16.5	40	44-64
		0.08	20
DL-alanine (63%) + L-glutamic acid (37%)	16.5	40	50-75
		0.1	50-20

2.1 *The effect of non-bridging ligands on electron transfer*

The effect upon reaction rate of changing the nature of ligands other than that in the bridging position have been studied. When $[(NH_3)_5CoOH_2]^{3+}$ is the oxidant and Cr^{2+} the reductant, the rate of reaction is increased if sulphate or pyrophosphate ions are present in the solution, and these ions are found[38] associated with the Cr(III). It has been shown conclusively that these added ions do not function as bridging ligands, since if $[(NH_3)_5 Co\,Cl]^{2+}$ is the oxidant, both Cl^- and the ion are found with the chromium after reaction. A recent study[39] has shown that the catalysis caused by the added ion is related to the order of stability of the complexes formed, the effectiveness falling off in the order EDTA > pyrophosphate > citrate > phosphate > fluoride > tartrate > thiocyanate > sulphate. The mechanism put forward for the reaction is $Cr^{2+} + L^{n-} \rightleftharpoons CrL^{(2-n)+}$ where L^{h-} is the non-bridging ligand and $CrL^{(2-n)+} + Cr^{3+}$

$$\rightleftharpoons \left[L\,Cr^{II} - \overset{H}{\underset{H}{O}} - Cr^{III} \right]^{(5-n)+}$$

$$\rightleftharpoons CrL^{(3-n)+} + Cr^{2+}.$$

It is possible for ligands to hinder electron transfer. Thus in the reaction between Tl(III) and Tl(I), added acetate and succinate ions decrease the rate, apparently by forming complexes with the Tl(III) which do not permit electron transfer through a bridging group[40].

3 REACTIONS BY THE OUTER-SPHERE COMPLEX AND BY UNCERTAIN MECHANISMS

Little new work has been done since the last comprehensive review of electron transfer by outer-sphere activated complexes. Wahl[41] has recently summarized results obtained for the rapid electron-transfer processes where the change in oxidation state takes place without a large change in the dimensions of the molecule. The results are given in Table 6.

Table 6 Comparison of experimental and predicted specific rate constants for electron transfer

Couple	theoretical k*	experimental k* (ref. 4)
$[Os(dipy)_3]^{2+}$, $[Os(dipy)_3]^{3+}$	10^5	1×10^8
$Fe(C_5H_5)_2$, $[Fe(C_5H_5)_2]^+$	10^4	2×10^6
MnO_4^{2-}, MnO_4^-	250	4×10^4
$[Fe(CN)_6]^{4-}$, $[Fe(CN)_6]^{3-}$	5	8×10^3

* l. mole^{-1}sec^{-1}

Marcus[4] has divided ions into three classes, ranging from class I (no change in bond lengths with change in oxidation state) to class III (large change) and has predicted that the rate of electron transfer should decrease in the order class I > class II > class III. The data given in Table 4 show that although the quantitative agreement is not good, the same trends are observed in both the experimental and predicted rates.

The system MnO_4^{2-}–MnO_4^- has been studied very thoroughly. The latest work by Wahl[42] shows that electron transfer is accelerated by

the presence of Cs^+ ion and it is suggested that a bridged activated complex is formed $[MnO_4–Cs–MnO_4]^{2-}$. Since it is assumed that the negative ions must be close for electron transfer to take place, the presence of the positive ion reduces the coulombic repulsion, just as anions act as mediators in electron transfer between cations.

One of the problems in separating the isotopic species in these rapid reactions is the exchange caused by the quenching technique used. Wahl[41] has discussed some of the more promising systems. Comparatively new methods (not limited to outer-sphere mechanisms) which do not involve separation of the reacting species, but which are of more or less limited application, are the measurement of the water exchange[43] by $[Cr(H_2O)_6]^{3+}$ in the reaction between $[Cr(H_2O)_6]^{3+}$ and $[Cr(H_2O)_6]^{2+}$, the measurement of the rate of racemization of optically active ions[44], and more recently, the use of nuclear or electron-spin resonance techniques[45, 46]. Recently a theory has been developed[45] which predicts that the nuclear magnetic resonance of a molecule or ion in the diamagnetic state would be interrupted by the large magnetic hyperfine field when it is converted to the paramagnetic state (the field arises from the unpaired electrons in the paramagnetic state).

If an ion is present in solution in both paramagnetic and diamagnetic forms, then the nuclear resonance line width observed is a measure of the lifetime of the diamagnetic ion. The method has been applied to Cu(I) and Cu(II) exchange (in hydrochloric acid)[45], and more recently[47] to the V(IV)–V(V) exchange. In the latter case it is found that no interaction occurs between V(V) and V(IV) at low acid and chloride concentrations, but that the interaction increases with both increasing H^+ and Cl^- ion concentrations. The electron transfer process is first order in V(IV), second order in V(V) and the specific rate constant $k = 1.5 \times 10^6$ 1.^{2} mole^{-2} sec^{-1} ($[Cl^-] = 3M$ and $[H^+] = 6.5M$). The mechanism proposed is $2V(v) \rightleftharpoons V_2(v)$, dimerization followed by $V_2(v) + V(IV) \rightleftharpoons V(IV) + V_2(v)$, electron transfer.

The function of chloride ion is either to provide chloride bridging or to give a reacting species which is a chloride complex. Some idea of the fast rates which can be determined by this method may be obtained from the results in Table 7.

Table 7 Specific rate constants of electron-transfer processes from EPR and NMR measurements

Couple	k(l. mole^{-1} sec^{-1})	Reference
V(IV)/V(V)	1.5×10^6	47
Cu(I)/Cu(II)	0.5×10^8	45
napathalene-naphthalenide ion	10^7 to 10^8	46

Some interesting explanations[12] have been advanced for observations made in the electron transfer between tris (1,10-phenanthroline)Co(II) and tris (1,10-phenanthroline)Co(III) complexes. It was shown by a tracer study that the rate laws depend on the anion present in solution; in potassium nitrate the rate is first order in Co(II) and first order in Co(III), but in potassium chloride the rate is first order in Co(III) but only half order (0.5) in Co(II). Furthermore, at any given concentration, the rate in potassium nitrate is two or three times greater than in potassium chloride solution. When the concentration of chloride ion is 0.05M, the rate is smaller than in the absence of Cl^-; as the concentration is increased the rate increases and the rate law dependence on the cobalt(II) complex becomes less than unity. To account for this, it is postulated that ion pairs are formed between $[Co(phen)_3]^{3+}$ and Cl^-, with the chloride ion in a position close to the metal, between the planes of the phenanthroline rings. To prevent the net transfer of energy during the electron transfer reaction, the chloride ion must move in the opposite direction, over a distance of several angstrom units. If this is energetically unfavourable electron transfer may be inhibited and if the process becomes sufficiently slow electron transfer may proceed by prior dissociation of the ion pairs. It is assumed that the greater size of the nitrate group ensures that ligand transfer takes place over a shorter

distance, with a correspondingly greater rate of electron transfer. The rate is not affected by changing to D_2O as solvent and, from the results obtained in acetone-water mixtures, some statements can be made about the form of the activated complex. As the acetone content of the solutions is increased, the rate first decreases then remains constant.

Since $\Delta F^{\ddagger}_{app} = -RT \ln K_2 + \Delta F^{\ddagger}_{r} + \Delta F^{\ddagger}_{rep}$, the only changes in $\Delta F^{\ddagger}_{r}$ will be in the energy necessary for rearrangement of the solvent molecules (the free energy necessary for rearrangement of the coordinated groups remains constant). An attempt was made to relate this to the viscosity of the solutions, and to show that an activated complex with the complex ions in direct contact fits the data best. The rate constant found (1.1 l. mole^{-1} sec^{-1} at 0°C, no added anion) is greater than would be expected for a complex containing ligands with a high crystal-field strength, and is due to the conjugated (conducting) system in the ligand.

In the case of the bridged complexes or the outer sphere complexes so far discussed (except for $[(NH_3)_6Co]^{3+}$ and Cr^{2+}), some definite statements could be made about the mechanism of the reaction, because at least one of the reacting ions undergoes substitution much more slowly than it does oxidation or reduction. For the many systems which do not fulfil this condition, conclusions are necessarily more limited, especially when the process involves the transfer of more than one electron, and for ions in high valency states there exists also the possibility of reaction through products of hydrolysis.

A good discussion of the difficulties involved in a system such as Fe^{2+}–Fe^{3+} is given by Taube[9], where in spite of intensive research, little is known about the mechanism for the electron transfer. Since the Fe^{3+} complexes are substitution labile, the form found for the rate law (rate $= k(FeX^{2+})(Fe^{2+})$, where X is a ligand such as Cl^- or F^-) does not necessarily mean that ferric ion brings the ligand into the activated complex and the geometry of the activated complex cannot be known. The theory of hydrogen atom transfer[15] in the redox process rests on observations that energies of activation for the reaction of Fe^{2+} with a number of different ferric complexes are very similar, possibly indicating a common mechanism, and that changing the solvent from H_2O to D_2O changes the specific rate constants by a factor of 2. Recent work[48] has shown that in the reaction of $[Co(NH_3)_5OH_2]^{3+}$ with $Cr^{2+}{}_{aq}$, where no hydrogen-oxygen bonds are broken, the rate constant changes by a factor of 3.8 in going from H_2O to D_2O, and the suggestion (based on arguments at least as sound as those for H-atom transfer) has been made that the process actually proceeds by a bridged complex.

Because Ce(IV) is considerably hydrolysed in aqueous solution, the reaction between Ce(IV) and Ce(III) has been interpreted as taking place through activated complexes such as $(Ce^{3+})(Ce(OH)_2{}^{2+})$, $(Ce^{3+})(Ce(OH)_3)$ and $(Ce^{3+})(CeOCeOH^{5+})$. The reaction is strongly catalysed[9] by F^-, $SO_4{}^{2-}$ and $H_2PO_4{}^-$, but not by Cl^-. A study of the reaction of Co(III) with Ce(III) in $HClO_4$ shows[49] that the reaction takes place by a path $CoOH^{2+} + CeClO_4{}^{2+} \rightarrow$, but that in the presence of F^- or $NO_3{}^-$, the rate increases due to alternate paths $CoOH^{2+} + CeF^{2+} \rightarrow$ or $CoOH^{2+} + CeNO_3{}^{2+} \rightarrow$. A detailed analysis of the experimental data shows that the added anion complexes with the Ce^{3+} not Co(III). The catalysis by F^- is much greater than by $NO_3{}^-$.

A recent series of papers[50] deals with the reaction of Tl(I) and Ce(IV) in the presence of Mn(IV) or $PtCl_4$. The reaction is found to be independent of the Tl(I) concentration and first order with respect to Ce(IV). Here the only Ce(IV) species considered is $Ce(OH)^{3+}$. A stepwise process for both $PtCl_4$ and Mn(IV) is postulated, involving a two-electron transfer:

$$Tl^+ + PtCl_4 \rightarrow Tl^{3+} + [PtCl_4]^{2-}$$

$$Ce(OH)^{3+} + [PtCl_4]^{2-} \rightarrow Ce(OH)^{2+} + [PtCl_4]^-$$

$$Ce(OH)^{3+} + [PtCl_4]^- \rightarrow Ce(OH)^{2+} + PtCl_4$$

$$Ce(OH)^{2+} + H^+ \rightarrow Ce^{3+} + H_2O.$$

The reaction is inhibited by $SO_4{}^{2-}$, but not by $HSO_4{}^-$ (cf. ref. 40). Since the observed rate law can be accounted by the above steps, the alternate possibility of an intermediate Tl^{2+} stage is not considered.

A number of other reactions have been investigated recently and are considered to take place in stages. The reaction of Ce(IV) and Cr(III) is believed to proceed by three one-electron steps:[51]

$$Ce^{IV} + Cr^{III} \rightleftharpoons Ce^{III} + Cr^{IV}$$
$$Ce^{IV} + Cr^{IV} \rightleftharpoons Ce^{III} + Cr^{V}$$
$$Ce^{IV} + Cr^{V} \rightleftharpoons Ce^{III} + Cr^{VI},$$

and the reaction between Ce(III) and Hg_2^{2+} also proceeds by a stepwise mechanism[52].

$$Co^{III} + (Hg^{I}\text{–}Hg^{I}) \rightarrow Co^{II} + Hg^{II} + Hg^{I}$$
$$Co^{III} + Hg^{I} \rightarrow Co^{II} + Hg^{II}$$

The very complex reaction between Sn(II) and Sn(IV) in sulphuric acid has also been studied. The reaction is assumed to take place by two paths and a number of bridged dimers formed from pairs such as $(SnO(SO_4)_2^{2-})$ $(SnOH^+)$ or $(SnOH(SO_4)^+)$ $(Sn(OH)SO_4^-)$ have been suggested. The complex formed may then be symmetrical and contain one Sn(II) and one Sn(IV) atom[53].

Table 8 Reactants in electron-transfer reactions[54]

Reacting couple	ΔH^* kcal mole^{-1}	ΔS^* e.u.	other H^+ dependence
$NpO_2^+ + NpO_2^{2+}$	10.6	—11.7	+1
$Pu^{3+} + Pu^{4+} + H_2O$	9.5	—13	0
$Pu^{3+} + PuO_2^{2+}$	4.8	—40.4	none
$2U^{4+} + UO_2^{2+} + 2H_2O$	32.8	+25.8	none
$NpO_2^+ + NpO_2^+ + H^+$	17.6	—22.2	—
$1\frac{1}{2}\,Np^{4+} + \frac{1}{2}\,NpO_2^+ + \frac{1}{2}\,NpO^{2+} + H_2O$	36.8	+32.1	—
$Pu^{4+} + Pu^{4+} + 2H_2O$	—	—	—4
$Np^{3+} + NpO_2^+ + H^+$	5.9	—31.2	none
$U^{4+} + CeOH^{3+}$	13.9	+ 6.2	—2
$U^{4+} + Pu^{4+} + H_2O$	24.3	+30.1	none
$U^{4+} + Fe^{3+} + H_2O$	21.4	+15.5	—2
$U^{4+} + Fe^{3+} + H_2O$	23.6	+25.4	—1
$Np^{4+} + Fe^{3+} + 2H_2O$	34.6	+52	none
$UO_2^+ + UO_2^+ + H^+$	9.2	—15.9	none
$PuO_2^+ + PuO_2^+ + H^+$	18.8	— 6.2	+2
$Np^{4+} + NpO_2^{2+} + H_2O$	24.7	+18.4	—3
$U^{4+} + PuO_2^{2+} + H_2O$	17.6	+ 3.4	—
$U^{4+} + PuO_2^{2+} + H_2O$	21.4	+18.1	—
$V^{3+} + PuO_2^{2+} + H_2O$	15.2	— 6.0	—2
$Ti^{3+} + Pu^{4+} + H_2O$	16.1	+ 3.7	none

3.1 *Electron-transfer reactions of the trans-uranic elements*

Electron-transfer reactions of the ions of U, Pu and Np are complicated because of the large number of hydrolysis products possible. For the reactions listed in Table 8, the processes are all one-electron changes but it is difficult to express the intermediates uniquely, or even when this is possible, their concentrations may not be known with any certainty.

Apart from the first three reactions, which do not involve the formation or breaking of metal-oxygen bonds, the activated complexes may be imagined as being formed from hydrated reactant ions by the prior loss or gain of one or more hydrogen ions. There will be a gain in those cases where metal-oxygen bonds are broken and a loss where such bonds are formed. It can be shown that entropies depend primarily on the charges involved and that electron transfer does not appear to take place over any great distance.

The rate laws are usually complex. For example, the rate expression for the reaction between Np(IV) and Np(V) is

$$k_1(NpO_2^+)^2(H^+) + k_2(Np^{4+})^{1.5}((NpO_2^+)^{0.5}((H^+)^{-2};$$

apparently the first term corresponds to a dimer which then exchanges rapidly with Np(IV). The reactions between Fe^{3+} and U^{4+}

and between U^{4+} and PuO_2^{2+} may both give rise to two different activated complexes and in each case the lower charged complex has the higher ΔH^* and ΔS^* values. In the reaction between Fe^{3+} and U^{4+} or Np^{4+}, the rates all have first order dependence on M^{4+}, Fe^{3+}, but differ in their hydrogen ion dependence. The hydrogen ion dependence may be explained by writing the process as

$$[M(H_2O)_4]^{4+} \rightleftharpoons [M(OH)_4]^{+} + 4H^{+} + e$$

In the overall reaction four hydrogen ions must be removed. If these are the only protons involved, then the environment of the metal in the activated complex should be somewhere between that in $[M(H_2O)_4]^{4+}$ and $[M(OH)_4]^{+}$. Formally identical reactions may proceed by different mechanisms at different hydrogen ion concentrations. The sign of the change in free energy for removing the proton will determine which of the activated complexes will be more favourable.

The hydrogen ion dependence may also take non-integral values as may be seen from Table 9 which gives the rate law dependences for the reaction of U(IV) and U(VI) as alcohol is added to the aqueous solvent[55]. Equations for

Table 9 *U(IV) — U(VI) rate law dependences*

EtOH (%)	U(IV)	U(VI)	n in $[H^+]^n$
0	2.0	1.0	—3.0
30	0.93	1.4	—1.74
60	0.87	1.08	—0.97
90	2.89	0.10	—1.06
100	0	2.70	—1.26

the proposed mechanism in water show that water takes part in the formation of the activated complex. In 90 to 100% ethanol the intermediates are apparently changed and are no longer UOH^{3+} and UO_2OH^{+}.

Not all reactions are so complex. The rate of electron transfer between U(IV) and Pu(IV) takes the form $k(Pu^{4+})(U^{4+})(H^{+})^{-2}$ and apparently an activated complex is formed between the two metal ions by prior loss of two protons. There do not appear to be any other minor paths for reaction, but sulphuric acid causes an increase in rate[56]. For more details, the reader is referred to one of the recent reviews[54].

4 NON-AQUEOUS SOLVENTS

The reaction between Pb(IV) and Ce(III) in acetic acid has been investigated in detail[57] because the system is simple and may act as a model for other reactions in non-aqueous solvents. Although no direct evidence has been obtained for the presence of Pb(III) in the solutions, the observations can be explained on the basis of a two-stage reaction:

$$Pb^{IV} + Ce^{III} \rightarrow Pb^{III} + Ce^{IV} \text{ (rate determining)}$$

and

$$Pb^{III} + Ce^{III} \rightarrow Pb^{II} + Ce^{IV} \text{ (rapid)}.$$

These workers have investigated some solvent and ionic effects which are of interest. They find, for example, that addition of benzene to the acetic acid solutions increases the rate of reaction, and log k_{obs} varies linearly with the reciprocal of the dielectric constant of the solution, as predicted by the Born equation, but with the product $Z_A Z_B = -1$. The addition of ethanol, on the other hand, increases the rate in proportion to the ethanol concentration and this is apparently due to complexing with Pb(IV), so that reaction takes place initially by the two competing paths $Pb^{IV} + Ce^{III} \rightarrow Pb^{III} + Ce^{IV}$ and $Pb^{IV}EtOH + Ce^{III} \rightarrow Pb^{III} + Ce^{IV}$, so that the rate is given by $k(Ce^{III})(Pb^{IV}) + k'(Ce^{III})(Pb^{IV})(EtOH)$. Solvent effects and preferential solvation of the reacting species by alcohol may also complicate the interpretation. When sodium perchlorate is added to the solution, the rate decreases and the observed variation of rate with concentration suggests that normal salt effects do not apply in solvents of low dielectric constant such as acetic acid. Addition of a salt such as sodium acetate causes complexing of the two metals, and the rate law has the form

$$k[A + B(NaOAc)^{1/2} + C(NaOAc)](Ce^{III})(Pb^{IV}).$$

The few results reported show that possible tests of theories concerning the solvent or salt effects may not be as easy to carry out as was once hoped.

Some work has recently been done on the Fe^{2+}–Fe^{3+} system in nitromethane[58] and the results obtained confirm earlier measurements in alcohols. The half time for the reaction in anhydrous nitromethane is of the order of

days and the rate is strongly dependent on water concentration, suggesting that the activated complex involves water in some way. In alcohols the variation is more complex and the rate passes through a maximum as the water content increases. The behaviour in isopropanol is different from that in ethanol, which is surprising, and has not been satisfactorily explained even in the most recent work[50].

Liquid sulphur dioxide, thionyl chlorides and even 100% sulphuric acid have been used as solvents for oxidation-reduction reactions[60]. In the reaction $SO_2 + SO_3 \rightleftharpoons SO^{2+} + SO_4^{2-}$ in sulphur dioxide, oxygen exchange occurs rapidly without sulphur exchange. This is interpreted as oxide-ion transfer with the weaker acid (SO_2) acting as a base towards the stronger (SO_3); for sulphur exchange to occur sulphur trioxide would have to act as a base towards the weaker sulphur dioxide. Sulphuric acid is an interesting solvent because of its high dielectric constant. Sulphur exchange in the systems SO_2–SO_3 and SO_2–H_2SO_4 has been investigated and found to be extremely slow (the half time for exchange is respectively 1100 days (132°C) and 20.5 days (137°C)).

5 PREPARATIVE APPLICATIONS OF ELECTRON TRANSFER PROCESSES

Electron-transfer processes offer a new means of preparing complexes of metals in substitution-inert oxidation states. The transfer of the bridging ligand to chromium has already been discussed above and an early unsuccessful attempt[61] was made to prepare the interesting $[(H_2O)_5CrSCN]^{2+}$ ion, by use of the reaction between $[(NH_3)_5CoNCS]^{2+}$ and Cr^{2+}_{aq}. It was postulated that remote attack of the ligand by Cr^{2+} would give rise to the intermediate $[Co–NCS–Cr]^{4+}$ (adjacent attack would give $[Co–N–Cr]^{4+}$, and after electron transfer, the chromium(III) complex should be $[Cr–S–C–N]^{2+}$. Unfortunately this complex is unstable and dissociation takes place, with subsequent reintroduction of a thiocyanate group to give the N-thiocyanate complex $CrNCS^{2+}$.

The method has also been applied[62] to the preparation of the ferri-*cyclo*hexane-1,2-diamine tetraacetic acid complex. The reaction between ferric xylenol orange and the tetraacetic acid is slow, but is catalysed by ferrous ion:

$$Fe^{II} + CHENTA \rightarrow Fe^{II}CHENTA$$
$$Fe^{III}XO + Fe^{II}CHENTA \rightarrow Fe^{II}XO + Fe^{III}CHENTA$$
$$Fe^{II}XO \rightarrow Fe^{II} + XO$$

(XO = xylenol orange, CHENTA = *cyclo*hexane-1,2-diamine tetraacetic acid).

These two examples show the possibilities of the method and it is likely that much more use will be made of this technique in the future.

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Trace-Ion Diffusion in Electrolyte Solutions

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1 INTRODUCTION

Trace-ion or single-ion diffusion is the name given to the movement of an ionic species in very low concentration through a uniform supporting electrolyte of much higher concentration. It is a section of the wider field of tracer diffusion which includes the study of both ionic and non-ionic species. When the trace ion is isotopic with one of the solution components, the term 'self-diffusion' is often used, but for the sake of generality, it will not be used in this review.

In the system described above, there is virtually no activity coefficient gradient and the diffusion potential is very small so that the net movement resulting from the unbiassed thermal motion of a single ionic species is observed. From a physical point of view, the diffusion process can therefore be thought of as proceeding as a statistical consequence of its Brownian motion. More formally, the free energy for such a process can be related to the entropy of mixing for ideal mixtures.

Much of the interest in trace-ion diffusion stems from the fact that the observed diffusion rates arise from this simple type of motion. It will be appreciated that the mobilities of ions as measured by other transport processes such as conduction and salt-diffusion arise from more complex causes. In each of these processes, the measured properties reflect contributions from both positive and negative ionic mobilities and are complicated by the presence of electrical and chemical potential gradients respectively. In trace-ion diffusion, however, the concentration dependence of the mobilities of the separate ions (in dilute solutions) can be related to a single long-range electrical effect. This 'relaxation-effect', as it is called, can thus be studied as an isolated property and the simplicity of this type of description may offer one avenue for extending the electrolyte transport theory to more concentrated solutions.

The movement of a trace species can be followed by chemical analysis in certain cases and more generally by the use of stable and radioactive isotopes. The use of radioisotopes is particularly convenient as the concentration of the trace species can be made extremely low ($\sim 10^{-10}M$ in some cases). Stable isotopes have not yet been employed in ionic studies but will probably be necessary in the case of such ions as Li^+ and NO_3^-. No precise experiment has been made to determine the difference in mobilities between isotopic ionic species but it is assumed to be very small. For example, Stokes, Woolf and Mills[1] studied the trace diffusion of ^{127}I and ^{131}I in various systems and found no difference in diffusion rate within the precision of their measurements ($\pm 0.4\%$). It might be added that diffusional movement in the liquid phase is conditioned chiefly by the size and shape of particles, not their mass and Baker and Pope[2] have demonstrated this in a recent study. Further, the hydration of small ions, where otherwise the effect would be greatest, reduces the relative mass difference considerably. From the foregoing, it will be

apparent that progress in this field has been very dependent on the availability of radioisotopes and almost all of the fifty or so papers on trace-ion diffusion have appeared in the last decade.

No comprehensive review of trace-ion diffusion in electrolytes has appeared previously and there has been no general tabulation of the associated data. As the values of diffusion coefficients obtained by the different experimental methods differ considerably in many cases, a large part of this review will be devoted to an assessment of the accuracy of all reported data. In order to do this effectively, those aspects of the procedures which may lead to erroneous values will be discussed in some detail.

2 EXPERIMENTAL PROCEDURES

Diffusional movement is usually reported in the form of a diffusion coefficient and for trace-ion diffusion this can be defined by Fick's Law. Two forms for unidimensional diffusion are given as under

and
$$-J_j = D_j \partial c_j/\partial x \qquad (1)$$
$$\partial c_j/\partial t = D_j \partial^2 c_j/\partial x^2 \qquad (2)$$

where J_j is the flux of trace-ion j, c_j is its concentration, x the distance-coordinate measured in the direction of diffusion, t the time and D_j the diffusion coefficient. Equation (1) is applicable to a steady-state diffusion process such as that approximated to in diaphragm cells and equation (2) where change of gradient with both time and distance is implied is used for free diffusion studies. These equations are, of course, suitably integrated under the boundary conditions applicable to the diffusion technique that is used. In salt-diffusion studies, the necessity of converting the measured integral coefficients to differential ones (D is concentration-dependent) and of considering reference frame conditions sometimes makes interpretation of the measurements difficult. In tracer diffusion studies, however, these effects become negligible and experimental evaluation of differential coefficients is in principle both accurate and simple.

In order to minimize the effect of convection, diffusion measurements in liquids usually require accurate control of temperature and the confinement of the process to fine capillaries of various kinds. The first requirement is fairly easily obtained but the second introduces other complications. The two commonest methods of measuring trace-ion diffusion coefficients have been based on the diaphragm-cell and the open-ended capillary. A capillary-cell was also used by Wang and Kennedy[3] in one series of measurements and other minor methods are mentioned in the literature but low precision and susceptibility to error excludes them from consideration here.

2.1 Diaphragm-cell method

This technique had been used for many years for concentration-diffusion studies and was first specifically adapted to trace-ion diffusion by Adamson[4]. It is a 'pseudo-steady' state method and therefore utilizes equation (1) for coefficient determination. A full account of the method is given by Robinson and Stokes[5]. The diffusion-cells are usually calibrated with accurate salt-diffusion data and provided Stokes's[6] magnetically-stirred cell is used, the method gives accurate coefficients of good precision (± 0.2 to 0.5%). Two sources of error warrant discussion; the first is related to ion-adsorption on the glass diaphragm and the second is concerned with the speed and manner of stirring.

The adsorption effect has been described by Stokes[6] and Nielsen, Adamson and Cobble[7]. Ion adsorption and the consequent formation of a double layer on the large glass surface of the sinter makes the measured diffusion process heterogeneous in so far as there is probably a contribution from surface diffusion. The effect naturally becomes more significant the lower the concentration and it is found that measurements below about $0.05M$ (for univalent salts) are subject to this error. The diaphragm-cell in its present form cannot be used therefore for measurements in dilute electrolyte solutions and data reported in the literature for concentrations $<0.05M$ will not be tabulated. This limitation effectively precludes use of the method for testing the limiting laws of electrolyte theory.

The stirring-rate effect was first reported by Nielsen, Adamson and Cobble[7]. They found that at high electrolyte concentrations their measured diffusion coefficients increased when the stirring speed was raised from the normal

rotation rate of 60 to 80 r.p.m. As their data for Na^+ in NaCl were in conflict with those obtained by Wang and Miller[8] with the capillary method, the effect of stirring speed was reinvestigated by Mills[9]. In this study, it was found that in a cell of Stokes's design (Nielsen, Adamson and Cobble's cell differed in several respects), there was no comparable effect when the stirring rate was raised from 60 to 110 r.p.m.

2.2 Open-ended capillary method

For brevity, this method will hereafter be referred to as the capillary method. It has also been called the capillary-cell method by some authors but this term is more properly reserved for a technique such as that used by Wang and Kennedy[3]. The method, which was developed specifically for tracer diffusion studies by Anderson and Saddington[10] in 1949, is particularly suited to radioisotopic work. The reader is referred to Wang[11] and to Robinson and Stokes[5] for general descriptions of the method. In theory, the method is an absolute one, but in practice some form of calibration has been found necessary because in many cases it has given inaccurate data. This appears to be due to the effect of convectional disturbances at the junction of the mouth of the capillary and the outer bath solution. Two separate effects can be distinguished.

The immersion effect occurs when a capillary is first lowered into the bath solution. Measurements of the convectional removal of radioactivity by this junction have been made by Krauss and Spinks[12], Mills and Kennedy[13] and Mills and Adamson[14], and a loss can occur ranging from 0.5 to 2% of the contents of the capillary. The effect can be minimized by leaving a large drop of solution on the capillary face and by very gradually lowering the tube at the time of immersion. If such precautions are adopted, the effect appears to cause a negligible error in the measured coefficients particularly with those variants of the technique in which only the activity remaining in the tube after a considerable period of time is measured, when the activity gradient should be fully re-established.

The other effect arises from the necessity of maintaining a flow of the outer bath solution across the capillary mouth. Equation (**2**) is applicable to capillary diffusion and one of the boundary conditions under which it is solved, can be expressed, $C_j = 0$ for $x > l$ where l is the length of the capillary. This condition is equivalent to the assumption that any labelled ion which diffuses through the plane of the capillary mouth does not recross it. Anderson and Saddington[10] and a few other workers did not provide such a flow and in these cases the measured diffusion coefficients would tend to be low due to the build-up of a concentration gradient of labelled species in the vicinity of the mouth. Wang[11] therefore introduced stirring by means of a slowly rotating paddle placed some distance below the capillary. However, this type of stirring appeared to give high results in some instances which were attributed to scooping out of the capillary solution by turbulent eddies and currents. Such an action is equivalent to shifting the plane where $C_j = 0$ a short distance down the bore of the capillary so reducing the effective length of the diffusing column. It has therefore been termed the 'Δl effect' by Wang[15] and he attempted to correct for it by adjusting his stirring speed so that capillaries of varying lengths gave the same diffusion coefficient. The effectiveness of his procedure was however reduced by the fairly low precision of capillary measurements (± 1 to 2%) at that stage of development. Later, during an investigation of anomalies between diffusion data from diaphragm-cells and capillaries, Mills[9] introduced an apparatus in which a controlled streamlined flow of solution was maintained across the face of the capillaries. Under these conditions the 'Δl effect' is minimized and by altering the flow rate, co-efficients in agreement with diaphragm-cell data could be obtained. Following this development, Mills and Godbole[16] calibrated their continual monitoring apparatus (see below) for flow rate against reliable diaphragm-cell data. Recently, Berne and Berggren[17] have reverted to turbulent stirring and have studied the 'Δl effect' by a more precise measuring technique. Finally, it should be stressed that not all the data obtained from paddle-stirred capillary studies disagree with diaphragm-cell values. Such factors as dimensions and shape of the apparatus, paddle speed, etc. apparently were occasionally of such character as to make the above effects negligible.

The low precision (±1 to 2%) of the capillary method as used by Wang[15], Mills and Kennery[13], Friedman and Kennedy[18] and others suggested that ion-diffusion coefficients could be measured by continuous monitoring of capillaries and he further proposed to eliminate

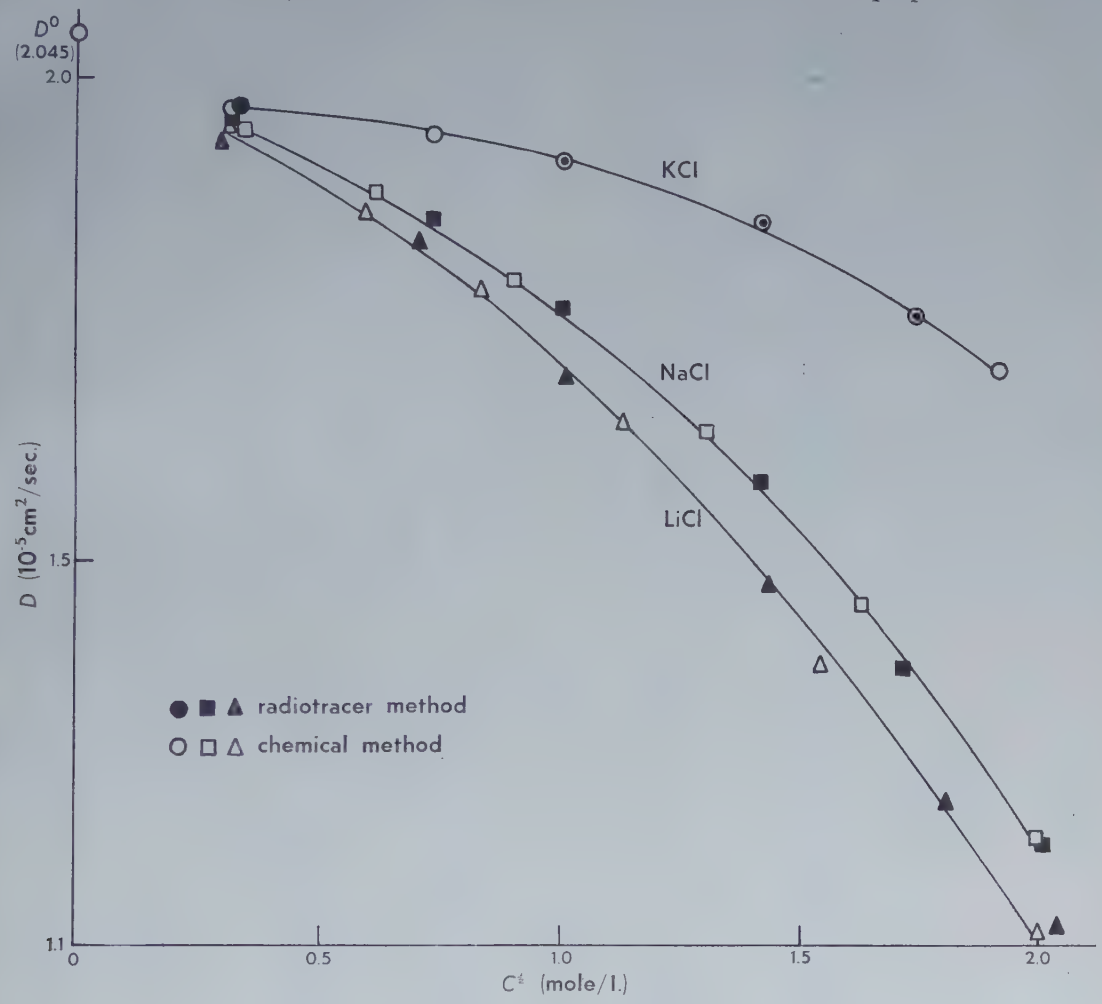

Figure 1 *Trace-ion diffusion coefficients of I^- ion in aqueous metal chlorides at 25°C. Data of Stokes, Woolf and Mills*[1]

was probably associated with the need to remove radioactive solution completely from the fine capillaries and to prepare reproducible counting samples. The precision of the method was greatly improved when Mills[19] suggested a procedure by which the radioactivity in the capillary could be continuously measured while diffusion is in progress. Measurements made by Mills and Godbole[20] indicate that precision of the order of ±0.2% can be obtained and this may be further improved if continuous recording is adopted. It should also be mentioned that Thomas[21] independently the 'Δl effect' by using a thin membrane over the capillary mouth. Although this very interesting method was used for measuring ion-diffusion in clay minerals, no trace-ion diffusion coefficients in electrolyte solutions have been reported.

3 ASSESSMENT OF DATA

Sources of error inherent in the experimental methods have been discussed in some detail so that a reasonably objective assessment of the accuracy of published data on trace-ion diffus-

ion can be given. Such an assessment is obviously necessary, when sets of conflicting data exist for several systems, when some capillary measurements are valid and others not, and when data from two different methods agree but it is subsequently shown that both sets are in error. The tabulation will be restricted to simple ions in homogeneous electrolyte solutions.

Data have been assessed on the basis of the following facts. The magnetically-stirred diaphragm-cell as introduced by Stokes[6], when properly calibrated, can be assumed to give accurate trace-ion diffusion coefficients when the concentration of the supporting electrolyte is greater than 0.05*M*. This is established by the agreement of salt-diffusion coefficients determined by such cells with optical and conductimetric data. Further, Stokes, Woolf and Mills[1] using such diaphragm-cells, have measured the tracer diffusion coefficients of iodide ion in a series of aqueous alkali chloride solutions. These measurements were made in separate laboratories and two distinct analytical techniques were used to analyse the tracer species, one a radiometric method and the other a potentiometric one. As illustrated in Figure 1, excellent agreement was obtained between the two sets of data, which showed an average deviation of only 0.4% over the whole range studied. Therefore, all measurements obtained with this type of apparatus will be accepted as accurate and be listed in Table 1.

Due to the inconsistency of the capillary method, the only data from this technique to be listed in Table 1 will be those that have been checked by diaphragm-cell measurements. This may be either by agreement between the two sets of data over a range of concentration or by adequate calibration of the flow rate. Other capillary data are listed in Table 2 and although probably accurate, must await confirmation. In cases where the method is suspect or where precision is poor, the values have been omitted.

4 TABULATION OF DATA

Table 1 *Trace-ion diffusion coefficients in aqueous electrolyte solutions at* 25°*C* (*data considered reliable*)

Trace ion	Supporting electrolyte	Method	Calibration procedure	Conc. (mole/l.)	D (10^{-5} cm^2/sec.)	Precision (%)	Reference
$^{131}I^-$	KCl	diaphragm	0.5*M* KCl → 0	0.1	1.966		
				1.0	1.918	±0.3	1
				2.0	1.850		
				3.0	1.758		
I^-	KCl	diaphragm	0.1*M* / 0.5*M* KCl → 0	0.1063	1.971		
				0.5002	1.945		
				1.000	1.918	±0.2	1
				1.980	1.854		
				2.996	1.759		
				3.669	1.703		
$^{131}I^-$	NaCl	diaphragm	0.5*M* KCl → 0	0.1	1.962		
				0.5	1.855		
				1.0	1.765	±0.3	1
				2.0	1.585		
				3.0	1.392		
				4.0	1.220		
I^-	NaCl	diaphragm	0.5*M* / 0.1*M* KCl → 0	0.1162	1.944		
				0.3784	1.882		
				0.8035	1.796	±0.2	1
				1.687	1.634		
				2.634	1.453		
				4.032	1.205		

Trace ion	Supporting electrolyte	Method	Calibration procedure	Conc. (mole/l.)	D (10^{-5} cm²/sec.)	Precision (%)	Reference
$^{131}I^-$	LiCl	diaphragm	0.5*M* KCl → 0	0.0977	1.956		
				0.488	1.832		
				1.021	1.692	±0.3	1
				2.036	1.476		
				3.250	1.248		
				4.176	1.118		
I^-	LiCl	diaphragm	0.1*M* / 0.5*M* KCl → 0	0.0882	1.934		
				0.3548	1.859		
				0.6930	1.784	±0.2	1
				1.263	1.645		
				2.389	1.392		
				4.010	1.109		
$^{38}Cl^-$	KCl	diaphragm	0.5*M* KCl → 0	0.1	1.961		
				0.25	1.975		
				0.5	1.963		
				1.0	1.955	±0.5	22
				2.0	1.907		
				3.8	1.780		
				4.0	1.774		
$^{36}Cl^-$	NaCl	diaphragm	0.5*M* KCl → 0	0.1	1.952		
				0.5	1.854		
				1.0	1.772		
				1.44	1.686	±0.5	22
				2.0	1.614		
				3.0	1.449		
				4.0	1.262		
$^{36}Cl^-$	NaCl	capillary	checked by (22)	0.03	1.975		
				0.05	1.96		
				0.1	1.94		
				0.5	1.85		
				1.0	1.77	±1.3	23
				2.0	1.61		
				3.0	1.44		
				4.0	1.24		
				5.0	1.06		
$^{38}Cl^-$	LiCl	diaphragm	0.5*M* KCl → 0	0.097	1.938		
				0.511	1.814		
				1.019	1.679	±0.5	22
				2.021	1.490		
				2.903	1.317		
				3.740	1.163		
$^{36}Cl^-$	HCl	diaphragm	0.5*M* KCl → 0	0.0935	1.985		
				0.5341	1.926		
				1.106	1.866	±0.5	24
				2.193	1.735		
				3.450	1.589		
				4.467	1.526		
$^{22}Na^+$	KCl	diaphragm	0.5*M* KCl → 0	0.1	1.322		
				0.16	1.320		
				0.3	1.325		
				0.6	1.315		
				1.0	1.311	±0.3	25
				1.4	1.300		

Trace ion	Supporting electrolyte	Method	Calibration procedure	Conc. (mole/l.)	D (10^{-5} cm²/sec.)	Precision (%)	Reference
				2.25	1.279		
				3.0	1.258		
				3.3	1.257		
				4.0	1.231		
$^{22}Na^+$	NaCl	capillary	flow rate calibrated (14), (9)	0.000225	1.335		
				0.00076	1.332		
				0.00102	1.327		
				0.00500	1.319		
				0.0101	1.312	±0.18	16
				0.0252	1.297		
				0.0286	1.298		
				0.0495	1.294		
				0.0575	1.289		
				0.0970	1.280		
$^{22}Na^+$	NaCl	diaphragm	$0.1M$ / $0.5M$ KCl → 0	0.16	1.295		
				0.23	1.294		
				0.3	1.289		
				0.4	1.283		
				0.5	1.279		
				0.6	1.283		
				0.81	1.247		
				1.00	1.234	±0.5	9, 14
				1.44	1.202		
				1.69	1.161		
				2.25	1.110		
				2.56	1.088		
				2.72	1.061		
				3.00	1.033		
				4.00	0.929		
$^{22}Na^+$	LiCl	diaphragm	$0.5M$ KCl → 0	0.1023	1.280		
				0.4817	1.244		
				0.9621	1.186	±0.3	26
				1.902	1.070		
				2.813	0.983		
				3.648	0.886		
$^{86}Rb^+$	KCl	diaphragm	$0.5M$ KCl → 0	0.1	1.994		
				0.5	1.961		
				1.0	1.952		
				2.0	1.897	±0.6	26
				2.56	1.865		
				3.0	1.842		
				4.0	1.759		
$^{86}Rb^+$	NaCl	diaphragm	$0.5M$ KCl → 0	0.1	1.961		
				0.5	1.927		
				1.0	1.845	±0.6	26
				2.0	1.721		
				3.0	1.543		
				4.0	1.348		
$^{86}Rb^+$	LiCl	diaphragm	$0.5M$ KCl → 0	0.0805	1.921		
				0.4931	1.844		
				0.9812	1.749		
				1.962	1.582	±0.6	26
				2.813	1.395		

Trace ion	Supporting electrolyte	Method	Calibration procedure	Conc. (mole/l.)	D (10^{-5} cm²/sec.)	Precision (%)	Reference
				3.648	1.260		
				4.011	1.208		
$^{134}Cs^+$	KCl	diaphragm	0.5*M* KCl → 0	0.1	1.981		
				0.5	1.957		
				1.0	1.951	±0.4	27
				2.0	1.897		
				3.0	1.810		
				4.0	1.707		
$^{134}Cs^+$	NaCl	diaphragm	0.5*M* KCl → 0	0.1	1.936		
				0.5	1.892		
				1.0	1.828	±0.4	27
				2.0	1.651		
				3.0	1.504		
				4.0	1.315		
$^{134}Cs^+$	LiCl	diaphragm	0.5*M* KCl → 0	0.0805	1.890		
				0.4025	1.801		
				0.805	1.744		
				1.610	1.607	±0.4	27
				3.221	1.351		
				3.990	1.216		
				4.025	1.208		
H^+	KCl	diaphragm	0.5*M* KCl → 0	0.1605	7.958		
				0.4806	7.934		
				1.002	7.729		
				1.016	7.675	±0.2	28
				1.712	7.244		
				2.883	6.423		
				3.629	5.936		
H^+	NaCl	diaphragm	0.5*M* KCl → 0	0.1090	8.011		
				0.5418	7.473		
				0.8124	7.053		
				1.442	6.552	±0.2	28
				2.271	5.726		
				3.570	4.316		
				4.545	3.460		
H^+	LiCl	diaphragm	0.5*M* KCl → 0	0.0892	7.789		
				0.4240	7.244		
				1.010	6.283		
				1.311	5.606	±0.2	28
				1.586	5.298		
				2.974	3.662		
				4.590	2.109		

Table 2 Trace-ion diffusion coefficients in aqueous electrolyte solutions at 25°C (data probably accurate but require confirmation)

Trace ion	Supporting electrolyte	Method	Conc. (mole/l.)	D (10^{-5} cm²/sec)	Precision (%)	Reference
$^{131}I^-$	KI	capillary	0.010	2.020		
			0.048	2.001		
			0.10	1.985		

Trace ion	Supporting electrolyte	Method	Conc. (mole/l.)	D (10^{-5} cm²/sec)	Precision (%)	Reference
			0.20	1.975		
			0.30	1.965	±0.75	13
			0.50	1.955		
			1.00	1.941		
			1.44	1.920		
			2.00	1.891		
			3.00	1.842		
$^{131}I^-$	LiI	capillary	0.061	2.004		
			0.125	1.991		
			0.237	1.910		
			0.500	1.847		
			0.760	1.795	±1.6	13
			1.134	1.694		
			2.00	1.540		
			3.34	1.268		
			4.10	1.220		
$^{131}I^-$	RbI	capillary	0.01	2.035		
			0.04	1.990		
			0.10	1.990		
			0.25	1.976	±1.1	13
			0.51	1.919		
			0.85	1.860		
			1.26	1.840		
			1.31	1.824		
$^{131}I^-$	HI	capillary	0.050	2.011		
			0.100	2.013		
			0.145	2.003		
			0.290	1.970		
			0.806	1.903	±1.0	13
			1.075	1.877		
			2.210	1.780		
			3.320	1.705		
			4.040	1.643		
$^{131}I^-$	NaI	capillary	10^{-4}	2.043		
			0.04	1.974		
			0.25	1.906		
			0.50	1.842	±0.7	29
			1.00	1.755		
			2.00	1.606		
			3.20	1.442		
			4.00	1.310		
$^{36}Cl^-$	$CaCl_2$	capillary	0.0705	1.89		
			0.282	1.72		
			0.803	1.60		
			1.41	1.42	±1.7	30
			2.68	0.907		
			4.02	0.447		
			5.36	0.159		
$^{86}Rb^+$	RbI	capillary	0.010	2.055		
			0.038	2.047		
			0.070	2.037		
			0.110	2.017	±0.7	13
			0.185	2.008		

Trace ion	Supporting electrolyte	Method	Conc. (mole/l.)	D (10^{-5} cm^2/sec)	Precision (%)	Reference
			0.370	2.004		
			0.640	1.995		
			0.927	1.993		
$^{204}Tl^+$	KCl	capillary	0.005	1.92		
			0.02	1.90		
			0.05	1.86	±1.2	31
			0.10	1.84		
			0.20	1.79		

5 DISCUSSION

In this section, the tabulated data will be correlated with such theoretical and semi-empirical descriptions as exist for the concentration-dependence of trace-ion diffusion coefficients. Relationships with other diffusional processes and fields of study will also be briefly discussed. The limiting diffusion coefficient for infinite dilution, D_j^0, will be considered only in conjunction with concentration-dependent equations. For many years, it has been possible to calculate these limiting coefficients from other electrochemical measurements by means of the Nernst equation. Their temperature- and viscosity-dependence have been adequately treated elsewhere[5, 32].

The theory of diffusional processes in solution can be divided into phenomenological and kinetic approaches. The phenomenological approach which is based mainly on the thermodynamics of irreversible processes is by far the most important one at the present time. Kinetic treatments use the Stokes-Einstein relation as a basis and are then elaborated by a rate-process theory such as that of Eyring[33]. The formulae derived in this manner include quantities which cannot be calculated accurately so that the approach is not very informative. In addition, most of the kinetic equations proposed for trace-ion diffusion (Wang[23], Ottar[34], Samoilov[35]) relate to the process at infinite dilution and much improvement is necessary before these treatments can be extended to consider concentration-dependence. Further discussion will therefore be restricted to the phenomenological approach.

5.1 Phenomenological description

The most significant contribution to the phenomenological interpretation was contained in the theory of irreversible processes in electrolytes published by Onsager and Fuoss[36] in 1932. This theory included a treatment of multi-component diffusion and in 1945 Onsager[37] pointed out that a relationship for the special case of trace-ion diffusion could be derived from it. The relationship is based on the operation of the relaxation effect and gives the concentration-dependence of trace-ion diffusion at very low ionic strengths. For brevity, we shall give Onsager's equation, expressed in practical units, and reduced to the case where only three ions are present. For a derivation of this relation from the equation of continuity for transport in electrolytes, the reader is referred to Adamson[38] and for the reduction to a practical formula to Gosting and Harned[39], or Robinson and Stokes[5].

The expression becomes, for aqueous solutions of 1:1 electrolytes at 25°C, with a univalent trace-ion,

$$D_j = D_j^0 \left[1 - 0.7816 c^{1/2} [1 - \{d(\omega_j)\}^{1/2}]\right] \quad (3)$$

where $D_j = RT\lambda_j^0 / F^2$ (Nernst Equation) and

$d(\omega_j) = \frac{1}{2}\{\lambda_i^0/(\lambda_i^0 + \lambda_j^0) + \lambda_k^0/(\lambda_k^0 + \lambda_j^0)\}$

with $\lambda^0_{i,j,k}$ the limiting conductances of trace and supporting electrolyte ions respectively, R the gas constant, T the absolute temperature, F the faraday and c the electrolyte concentration.

The experimental testing of equation (3) has had to await the development of the capillary method to the point where it has a precision of a few tenths of a per cent. The diaphragm cell is unsuitable for such measurements for the reasons outlined earlier.

The continuous monitoring method developed by Mills and Godbole[16] is now capable of precision of the order of 0.2% and recent data allow a valid comparison to be made. In Figure 2 results are shown for the diffusion of

sodium ion in dilute sodium chloride solutions and it will be seen that below 0.001*M*, the data appear to follow the limiting slope.

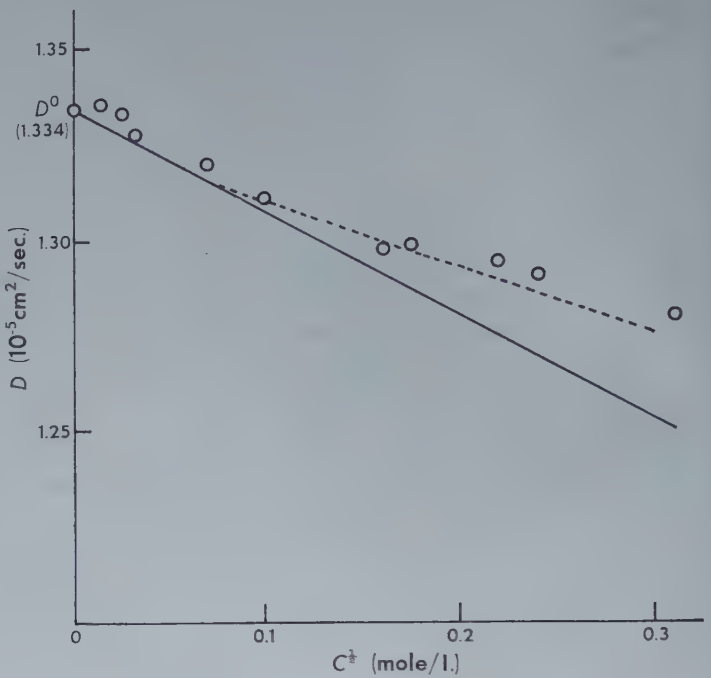

Figure 2
Trace-ion diffusion coefficients of Na^+ ion in aqueous NaCl at 25°C. Data of Mills and Godbole[16]. ———, Onsager limiting law; - - - - - -, ion size extension (a = 4Å).

5.2 Empirical extensions

If the D_j values for the various ions given in Table 1 are plotted against $c^{1/2}$ (see, for example, Figures 2 and 3) it will be observed that in all cases, the resultant curves approach the limiting slopes from above. It is probable that departure from these slopes is related to the necessity for introducing a correction for ion size into the limiting equations. Successful modifications of this type have been made to theoretical equations describing other electrolyte transport processes but there has been no analogous treatment for the case of trace-ion diffusion. However, it is perhaps worth while to examine the semi-empirical correction to the limiting law which can be made by including the ion size parameter, *a*. The limiting equation now becomes

$$D_j = D_j^0 - Ac^{1/2}/(1 - Ka) \qquad (4)$$

where K is the reciprocal of the mean radius of the ionic atmosphere.

The dashed line in Figure 2 shows the effect of such a modification where *a* has been given a value of 4Å. (this value is reported by Robinson and Stokes[5] to give the best fit with experimental data from conductivity studies). Agreement with the experimental data to 0.05*M* concentration indicates that this assumption is probably correct. Further, it has been shown by Mills[26] that the spacing of the coefficients for various ions at 0.1*M* can be semiquantitatively accounted for in terms of the limiting slopes and ion-size values, even though this concentration is at the extreme limit of applicability of the equation.

A discussion of trace-ion diffusion coefficients in concentrated solutions (>0.1*M*) must necessarily be qualitative in nature, in the absence of a theory to account for the phenomena at these concentrations. However, certain interesting facts emerge from an examination of the considerable amount of available data.

It is instructive to look at the data over the whole concentration range for Na^+ diffusing in NaCl as shown in Figure 3. The limiting slope and the ion-size correction curve are included. The experimental curves are typified by a concave curve leading to the limiting slope and Nernst value at low concentrations (<0.1 *M*), fairly constant coefficients rising above the curve for equation (4) at moderate concentration (0.1 to 1*M*) and then falling off rapidly below the curve for equation (4) at high concentrations (>1*M*). Qualitative explanations for this behaviour have been offered by several authors.

The close relation between diffusion and viscosity in liquid media is well known and finds expression in such descriptions as the Stokes-Einstein equation and Walden's Rule. Crude correction such as simple multiplication of ion-diffusion coefficients by the solution viscosity does not give the constant products required by the above equations. This might be expected since these relations were only meant to apply to large, uncharged particles in pure solvents. Nevertheless, in all cases, the D_j *vs.* $c^{1/2}$ curves for a given ion in solutions of differing viscosity are brought much closer together by the above treatment. Another important effect which invalidates corrections involving the macroscopic viscosity is the non-uniformity of this property in an electrolyte solution. From a microscopic point of view, the local viscosity in the close vicinity of an ion in an aqueous solution is probably very different from that in the bulk of the solution. This situation arises because of the existence of a structure in aqueous solutions which when coupled with the polar nature of the water molecule causes distortion of the fluid in close proximity to an ion.

(0.1 to 1M) in Figure 3. Wang[32] has discussed the effect of structure distortion of the electrolyte in terms of the distances between oppositely charged ions in this range of concentration. When such ions approach one another, it becomes increasingly hard for the intervening solvent to maintain its tetrahedrally coordinated structure. This distortion decreases the local viscosity and the mobility of the diffusing ion is enhanced. The balancing of this effect with that of relaxation and other retarding effects would account for the observed plateau but eventually the latter become dominant and the diffusion rate decreases again. This can be called the primary effect.

A secondary effect has also been distinguished by Wang[32], based on evidence from the temperature-dependence of the limiting diffusion coefficients of ions. These values suggest that unhydrated ions such as Rb^+, Cs^+ and I^- at infinite dilution, cause a greater distortion in the water structure in their immediate vicinity than do hydrated ions such as Li^+ and Na^+. Consequently, at concentrations where the plateaux are observed, the relative distortion around hydrated ions will be greater and the

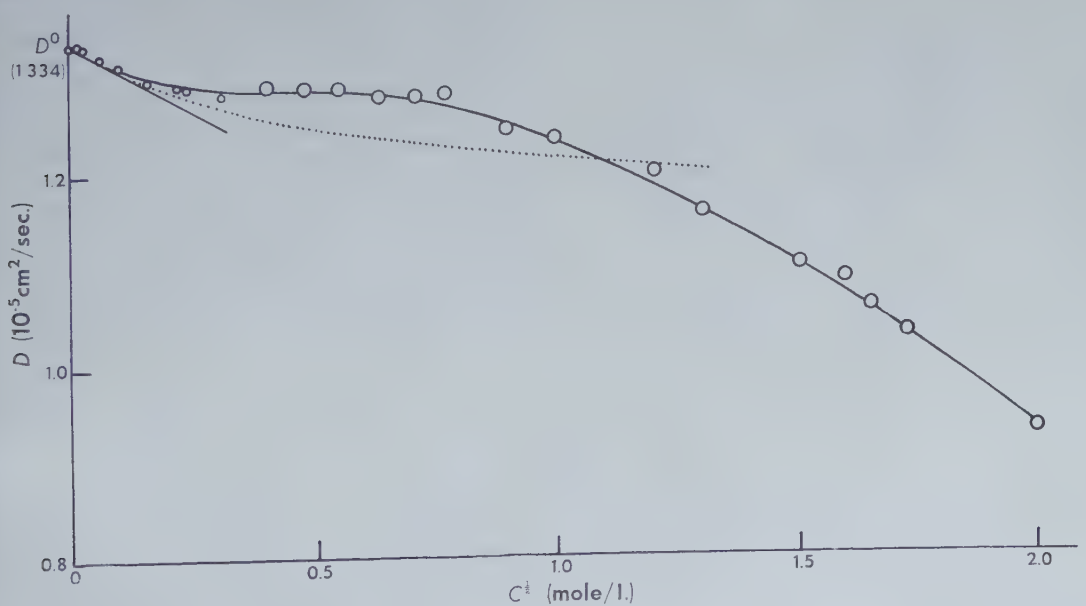

Figure 3 *Trace-ion diffusion coefficients of Na^+ ion in aqueous NaCl at 25°C. Data of Mills* et al.[9, 14, 16]. ———, *Onsager limiting law;* . . . . ., *ion size extension.*

This concept of local viscosity can be invoked to explain the plateau region in the D_j *vs.* $c^{1/2}$ curves in the concentration region

effect will be more marked. The available evidence appears to support this view as, for example, the shape of the I^- and Na^+ curves in

Figures 1 and 3 but more diaphragm-cell data on highly hydrated ions are needed.

Finally, it may be remarked that although refinements in the viscosity term may give semi-empirical equations agreeing with experimental data any eventual theoretical treatment will require the use of more fundamental factors which influence the parallel processes of viscous flow and diffusion.

5.3 Relation to other diffusion processes

5.3.1 Salt diffusion

In the case of a binary salt, it is possible to calculate the differential salt coefficient and the two isotopic trace-ion (self-diffusion) coefficients at any specified concentration. It would be of considerable interest to find relationships between these three coefficients. At infinite dilution they are of course simply related through the Nernst equation. At finite concentrations however the relationship is complicated by the different electrical effects acting on the two processes and by the presence of a thermodynamic term in one of them.

5.3.2 Mixed diffusion

Trace-ion diffusion has a direct connection with other multicomponent diffusion processes. This relationship is perhaps best illustrated by reference to a specific example, for instance the three-component system NaCl-KCl-H_2O. The flows in such a system are described by the equations:

$$J_1 = -D_{11}(\partial c_1/\partial x) - D_{12}(\partial c_2/\partial x) \quad (5)$$
$$J_2 = -D_{21}(\partial c_1/\partial x) - D_{22}(\partial c_2/\partial x) \quad (6)$$

where NaCl is component 1 and KCl component 2. D_{11} and D_{22} are termed main and D_{12} and D_{21}, cross-term diffusion coefficients.

These equations express the fact that in such systems there is interaction of flows and therefore, in general, the Fick's Law equations which describe binary diffusion are inadequate. Trace-ion diffusion is a limiting type of three-component system, as in this instance the concentration of the trace component is extremely low. From equation (**5**), it can be seen that as $c_1 \rightarrow 0$ then $D_{12} \rightarrow 0$ also. D_{11} now becomes the trace-ion diffusion coefficient of Na^+ in KCl and, under these special circumstances, Fick's Law can be used to describe the diffusion of the trace species. Knowledge of trace-ion coefficients is therefore of considerable interest in studies of multicomponent diffusion as is shown for example in the work of O'Donnell and Gosting[40].

5.3.3 Polarography

In polarographic studies, trace-ion diffusion coefficients are of interest in testing the various kinetic equations which describe the processes taking place at the dropping-mercury electrode. Wang[41] has measured the trace-diffusion of divalent ions specifically to test these equations, and his results for Zn^{2+} and Pb^{2+} support the form of the Strehlow-von Stackelberg equation. Conversely, polarographic techniques have also been used to measure trace-ion coefficients. However, the large inaccuracies inherent in the polarographic method, as at present applied, completely excludes the data obtained from being of use in the fundamental studies which have formed the subject of this review.

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Glycosidases

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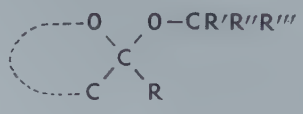
1 INTRODUCTION

The preparation of a general review of a specialized scientific subject is often a disillusioning experience for a writer. He is brought face to face with the question of whether the topic that he is proposing to discuss is in fact a legitimate specialization or merely a convenient pigeonhole for a body of not very closely related facts. In view of the somewhat sinistral relationship between chemistry and biochemistry, these difficulties are particularly apt to arise in the discussion of biochemical subjects; thus, the carbohydrates form a classically coherent chemical genus, but there is no *a priori* reason why the hydrolytic enzymes acting on them should be closely related in either constitution or mechanism of action. On the other hand it is equally plausible that all the enzymes that we classify on the basis of their chemically different substrates as glycosidases, peptidases, esterases, phosphatases and so forth, do in fact possess a common mechanism of action. The unity that can be imposed on the glycosidases is therefore chemical and not biochemical in its nature.

The glycosidic linkage will be taken as the hemiacetal bond in the following formula:



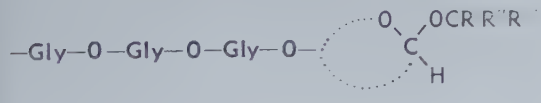
(R = H for aldose sugars and CH_2OH for 2-ketose sugars; the group CR′R″R‴ is the *aglycone*), and the glycosidase catalyses hydrolysis to the parent sugar and the alcohol R′ R″ R‴ COH. The ring system is that of a monose sugar (unsubstituted or further substituted) in the conventional Haworth formulation and the only ring systems for which enzymic decomposition has ever been demonstrated are the five-membered (furanose) and six-membered (pyranose) rings. The examples given in Figure 1 illustrate the principles of naming. The ring systems are numbered as shown and whenever the nature of the ring is certain from the context, carbon atom 2 (abbreviated C2) will be referred to without further definition.

The peculiarities of the glycosidic linkage that make it of interest in the study of enzymic mechanisms are these:

(1) An asymmetric carbon atom forms part of it, so that any configurational changes (or lack of them) found in biochemical transformations of the glycosidic bond may favour or rule out certain chemical mechanisms for the action of the enzyme. It is well known that only certain types of rupture of bonds to asymmetric carbon will change the absolute configuration about that atom.

(2) The asymmetric carbon atom itself forms part of a sterically complex system (the sugar ring) containing other asymmetric atoms. This ring can be modified by substitution and

when the substituting radical is another sugar radical (glycosyl unit) united to the present ring by a glycosidic linkage, the first unit acts as the aglycone. This substitution can be repeated until a chain of glycosyl units (Gly in the formula below) is built up:



Since it is possible in theory to make glycosides with any desired degree of resemblance to a glycoside known to be split by a given enzyme, we have very sensitive means of testing the *specificity* as well as the mechanism of hydrolytic enzymes. Thus if we know that an enzyme splits the glycosidic linkage in β-D-glucopyranosides we can set out to discover whether the changes shown in Figure 2, i.e. inversion of H and OH about C4 in the galactopyranoside, elimination of the CH_2OH on C5 in the xylopyranoside, O-methylation on C2 in the 2-O-methylglucopyranoside, are compatible with action of the enzyme on the glycosidic linkage.

(3) The aglycone radical, which has been noncommitally written as CR′R″R‴ may be anything from a simple alkyl or aryl radical to another long chain of glycosyl residues. Besides the sharp specificity which all glycosidases exhibit for the glycosyl residue, almost all show some degree of specificity for the aglycone; frequently the specificity is quite as sharp for both moieties. Depending on the nature of glycosyl unit and aglycone, certain types of glycosidic linkages can be distinguished, and these are shown in Figure 3 using the β(1→4) glycosyl linkages typical of cellulose for illustration. Not only are the various types of aglycone sterically distinct but, for a given glycosyl unit, the chemical nature of the aglycone will determine the availability of electrons

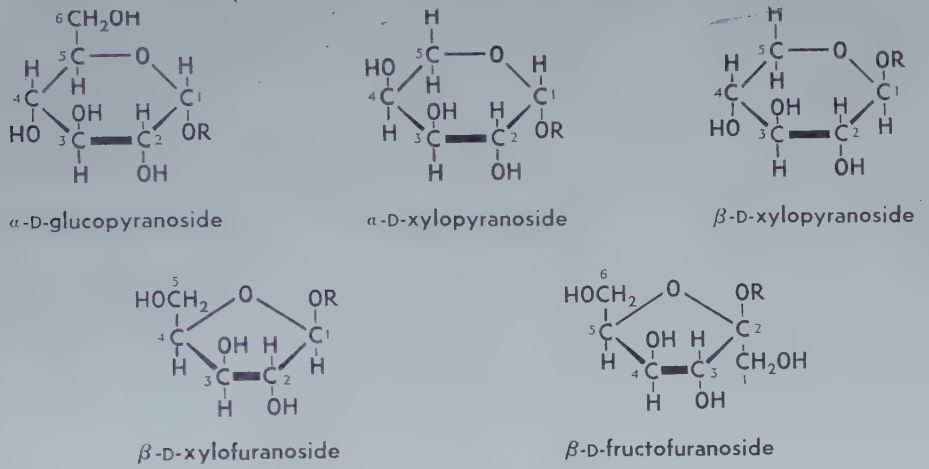


Figure 1

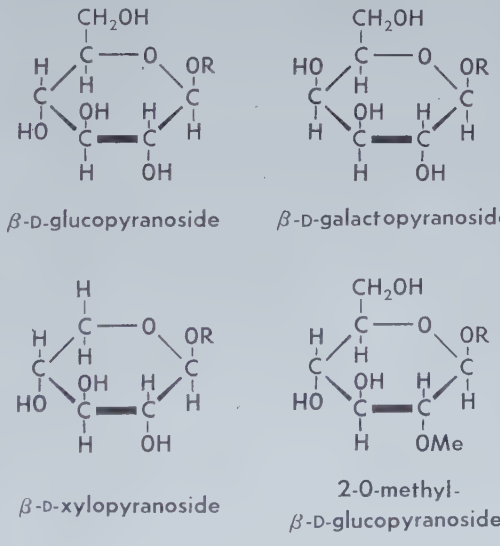


Figure 2

to the glycosidic linkage and the free energy yield from its fission. The nature of the aglycone can thus exert an influence on the type of reaction and the nature of the reaction products.

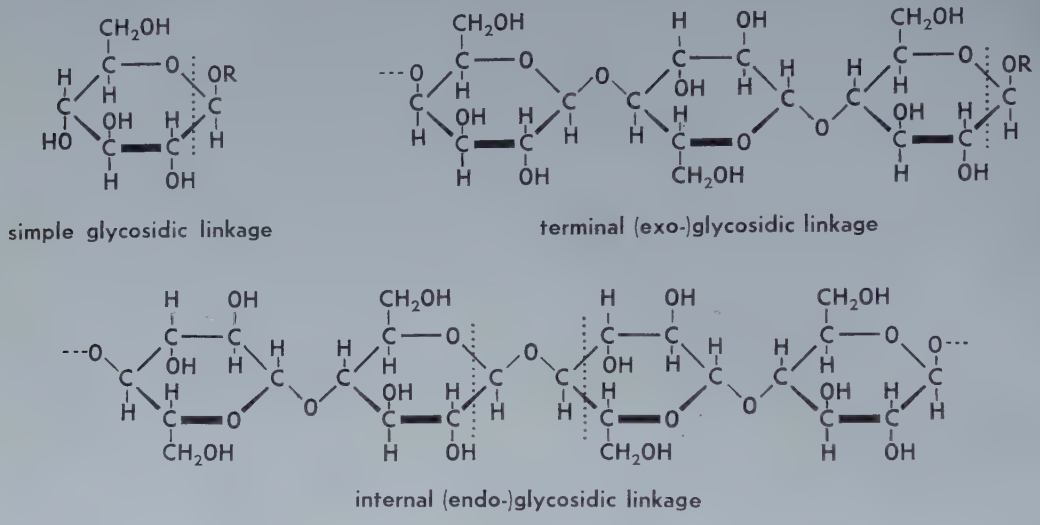


Figure 3

The introduction above amounts to a definition of a glycosidase somewhat wider than is usual, the customary definition being an enzyme that splits terminal linkages only, the aglycone not being carbohydrate in nature. But since all the evidence goes to show that differences in mechanisms are not centred round the question of whether the aglycone is carbohydrate (one or many glycosyl residues) or some other radical, this definition does not appear to delineate any biochemically distinct region. Since alternative names covering the whole range of enzymes hydrolysing the glycosidic linkage, e.g. 'carbohydrase', do not imply specificity for this linkage, the word 'glycosidase' has here been expanded in meaning to carry this implication.

2 SOME OBSERVATIONAL DATA

Glycosidases have been isolated from every form of life both in number and variety too great to warrant detailed citation. They range in frequency of occurrence from the universal amylases (starch-hydrolysing enzymes) to an enzyme of such scattered distribution as α-mannosidase, which Conchie, Findlay and Levvy[1] have shown to attain its highest natural activity in the epididymis of the rat. The metabolic function of glycosidases is frequently obscure and this observation is just as applicable to the well-known and widely-distributed enzymes as to the "waste-product" enzymes secreted by many micro-organisms.

2.1 *The point of rupture of the glycosidic linkage*

The glycosidic linkage presents the two possible points of rupture shown in Figure 4 and a choice between them can be made by the use of isotopes.

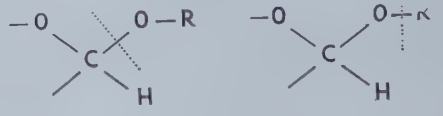


Figure 4

In an uncomplicated hydrolytic system (Gly OR + HOH → GlyOH + ROH) where OR is replaced by OH, the use of a glycoside with an enhanced proportion of $C^{18}OR$ will give the normal ratio of oxygen isotopes in the

resultant sugar if mechanism 1 is operating, but an increased ratio if mechanism 2 is operating; the ROH moiety will give the increased ratio for mechanism 1 and the normal ratio for mechanism 2. The reverse experiment with a normal glycoside and water enriched in $H_2{}^{18}O$ will give opposite results.

This technique was first applied to glycosidases by Bunton *et al*[2] and by Koshland and Stein[3, 4] and the results showed unequivocally that invertase (β-fructofuranosidase) as well as α- and β-glucopyranosidases brought about fission of the C–OR and not of the CO–R bond. Halpern and Leibowitz[5, 6] and Mayer and Larner[7] have shown that α-amylase and β-amylase both break the C–OR linkage between two of the $\alpha(1\rightarrow4)$-linked glucose residues of starch as shown in Figure 5. Eisenberg[8] has

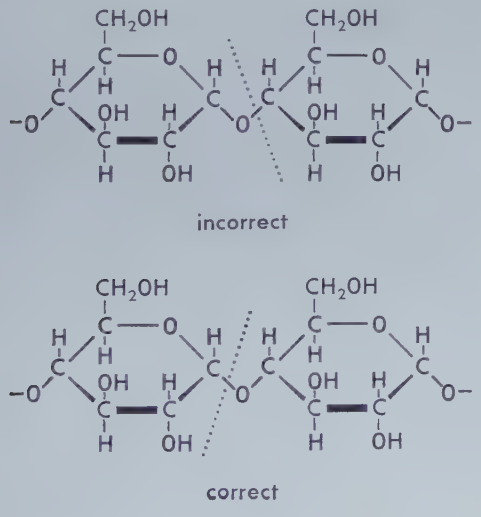


Figure 5

shown that both 1-O-benzoyl β-glucuronic acid and *p*-menthyl β-glucosiduronic acid (see Figure 6) are split as shown in Figure 5 by calf-liver β-glucuronidase, although it appears doubtful whether the enzymic hydrolysis of the two substances takes place by the same mechanism; certainly the non-enzymic hydrolysis does not.

It would appear that enzymic cleavage of carbohydrates generally takes place at the glycosyl-O bond and not at the O-aglycone bond[4] This conclusion fits in with a great deal of observation and theory and will be explained as a particular instance of Koshland's[9] generalizations to be discussed later; if this is so it is to be expected that the conclusion above is not necessarily true and certain types of enzyme (perhaps hypothetical) could split the O-aglycone bond.

2.2 *Configuration of the products of enzyme action*

One of the best-known antitheses in biochemistry is that between α- and β-amylase. The source of these names lies in the fact that the

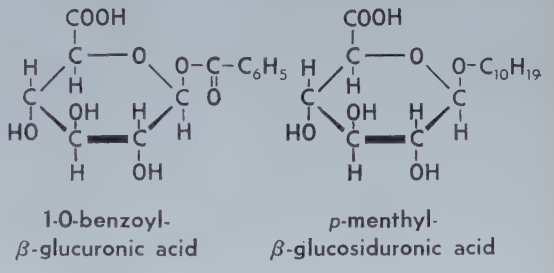


Figure 6

new bond formed by the action of these enzymes to replace the ruptured α-glycosidic linkage of starch is in the α-configuration for α-amylase and the β-configuration for β-amylase. This behaviour may be illustrated as in Figure 7 for α- and β-amylases which would both act on maltotriose (the enzymes vary widely in their specificity according to their source). These conclusions were originally drawn from observations on the course of the change in the optical rotation of solutions of starch during enzyme action. In some cases this is still the only method available, since sugars released in either the α- or β-configuration will eventually be converted into the same equilibrium mixture by mutarotation if their solutions are allowed to stand. Where the effect of enzyme action is to convert the old glycosidic linkage into a new one (transferase reaction), the new glycoside is a semi-permanent record of the configuration of the primary product of enzyme action.

Enough glycosidases have now been investigated to make it certain that the type of behaviour shown by β-amylase is exceptional; the general rule is that the configurations of substrate and product are the same. Apart from

β-amylase itself, the only glycosidases for which inversion of configuration seems to be well established are the amyloglucosidases ("glucamylases") such as iaka-amylase B

Since testicular hyaluronidase gives normal-type cleavage of the same glycosidic linkage it seems that apparent similarity of action is no guarantee of identity of product. It may well

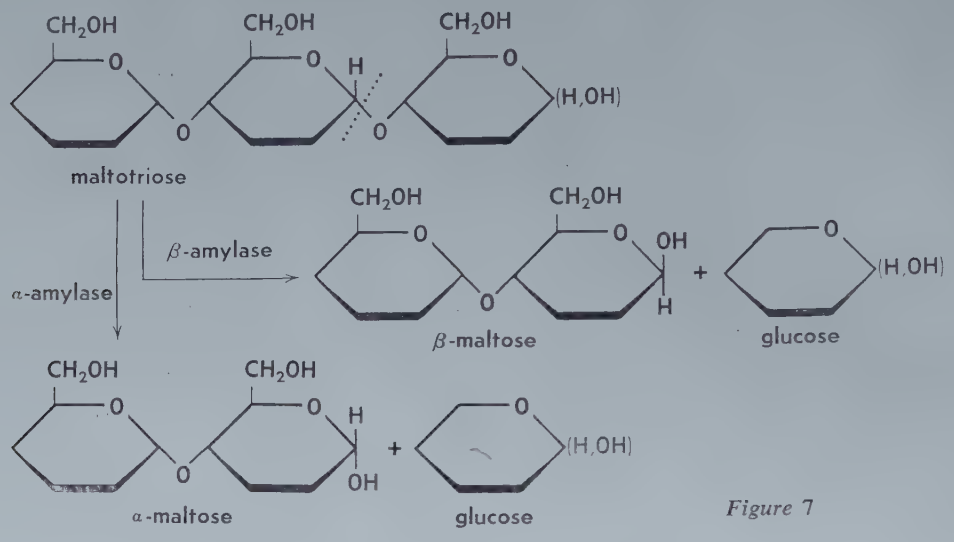


Figure 7

which remove a glucose rather than a maltose unit from the non-reducing end of a starch chain.[10]

It might be anticipated that the cleavage of a glycosidic linkage by a glycosidase will lead to the formation of a new bond in either the same or the inverse configuration; the work of Zinker, Mayer and Hoffman[11], although still not paralleled in any other system, shows that this is not always the case. A variety of hyaluronidases from bacteria break up the polysaccharide, hyaluronic acid, which has a chain

be that checking the products of other partly-investigated glycosidases that appear to conform to well-known types would reveal similar anomalies.

2.3 *Hydrolysis and transfer*

Many glycosidases, in suitable circumstances, give products which are strikingly anomalous if they are considered to be simple hydrolytic enzymes. If hydroxylic compounds other than water, e.g. aliphatic alcohols, are present they will form glycosides for which these com-

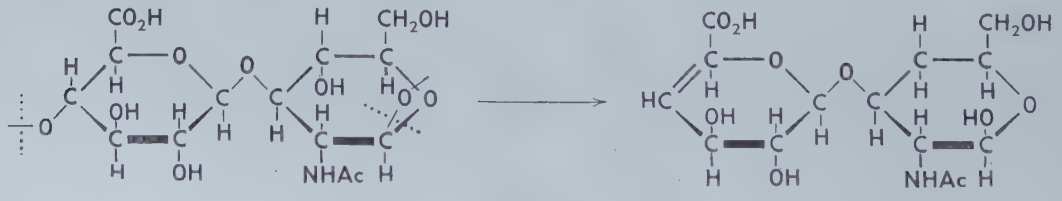


Figure 8

molecule made up of alternate repeating units of N-acetylglucosamine and glucuronic acid, into an unsaturated disaccharide as shown in Figure 8.

pounds provide the aglycone. Often new glycosides are formed in which the original glycoside or the sugar liberated by hydrolysis acts as aglycone. Such reactions are 'transfer re-

actions' and the enzymes carrying them out are 'transferases'.

The first reaction to such observations is to consider that the 'transferase' is an impurity in the hydrolysing enzyme ('hydrolase'). However an increasing number of cases in which the two activities proved inseparable, their proportion remaining constant through all attempts at purification, provide convincing evidence that the transferase and hydrolase could be considered as competing activities of the same enzyme.

$$\text{GlyOR} + \text{HOH} \rightarrow \text{GlyOH} + \text{ROH}$$
$$\text{GlyOR} + \text{QOH} \rightarrow \text{GlyOQ} + \text{ROH}$$

Finally, when the use of paper chromatography had made it possible to resolve complex mixtures of related sugars, Bacon and Edelman[13] showed that invertase formed intermediates during its action by transferring fructose residues to sucrose. Since these were later broken down by the enzyme the final reaction products were the expected glucose and fructose; nevertheless the reams of computation that had been devoted to the kinetics of this supposedly simple hydrolytic enzyme have now become of historical interest only. No investigator would now think of giving even a preliminary account of a new glycosidase without testing for the possible formation of transient intermediates during its action on a suitable substrate.

(1) Only hydrolases which split the glycosidic linkage with retention of configuration are capable of transferase action. Thus Okazaki[10] could not demonstrate transfer to any acceptor by taka-amylase B; while Sawai[14] showed that the apparently very similar "maltase" (in both cases enzymes removing the terminal non-reducing glucose residues from starch grains) of *Candida tropicalis* transferred glucosyl residues to a wide variety of acceptors with retention of configuration.

This restriction of transfer to enzymes that retain the configuration of the original donor does not apply to enzyme types other than the glycosidases that can transfer glycosyl residues. The biochemistry of the uridine diphosphate (UDP) transglycosylases has recently been reviewed by Hassid, Neufeld and Feingold[15] and they cite a number of instances in which the α-linked glycosyl residues of the UDP-glycoses are transferred to give new linkages in both the α- and β-configurations. Thus an enzyme from mung beans catalyses the transfer of a glucosyl residue from UDP glucose to give poly $\beta(1\rightarrow3)$-D-glucose (callose) as shown in Figure 9.

Although the systems involved in glycosyl transfer are formally so similar that it is permissible to wonder whether the separation of glycosidases from the UDP transglycosylases is more than another instance where related en-

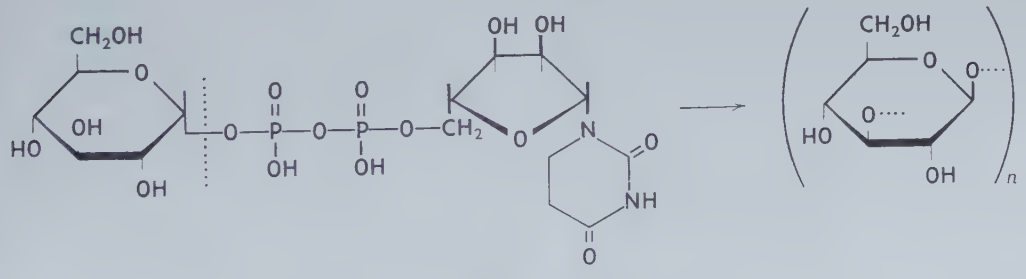


Figure 9

The substrate providing the glycosyl group for transfer is usually called the "donor" and the molecule to which it is transferred, the "acceptor". For hydrolase action, water is the acceptor; for most transferase actions water and some other substances are competing acceptors.

The following general points may be noted about transferase action.

zymes have been put in separate categories on purely chemical grounds, the variability in the configuration of the transfer products produced by the latter, means that the distinction may well be justified and that there is a profound difference in reaction mechanisms.

(2) Retention of configuration is a necessary but not a sufficient criterion for transferase action. Thus no true α-amylase has ever been

shown to have transferase action. This is not necessarily associated with its action as an endoglycosidase, since other endoglycosidases such as testicular hyaluronidase[12] and the Q-enzyme of amylopectin synthesis[17] are far more active as transferases than as hydrolases.

(3) Although there are many hydrolases that have never been demonstrated to possess transferase action, it is much harder to find examples of pure transferase action. Perhaps the most authentic case is the D-enzyme from the potato[18, 19] which has been shown by the use of isotopes to transfer polyglucosyl chains from starch to glucose without any trace of hydrolytic products.

(4) Transfer can only take place within the limits of what is thermodynamically possible. The free-energy change during the reaction remains the driving force. Where both donor and transfer products are substrates for the enzyme an equilibrium will be set up; when there are several competing acceptors, this equilibrium will be far more complex. A series can be written Gly-OAryl → Gly-OFuranosyl sugar → Gly-OPyranosyl sugar → Gly-OAlkyl → Gly-OH (free sugar) in which transfer from a higher member of the series to the aglycone of a lower member will normally be almost complete in the presence of an enzyme of suitable specificity. This implies that where the specificity allows, the normal final product of enzyme action will be the free sugar with other transfer products as intermediates.

Although the idea of different degrees of "energy richness" in glycosidic linkages can be defended to some degree on chemical grounds by invoking different degrees of electron-withdrawal from the bonds involved[20], it must be remembered that this concept is no more than a convenient mnemonic, like that of the "energy-rich phosphate bond". It provides a general indication of the direction and extent of reaction in simple cases and no more. In more complex cases such as the building of polymeric chains by transfer from simple glycosides, e.g. the building up of dextran (predominantly poly-α-glucosyl(1→6)) from sucrose, it is quite inadequate, although in theory a complete knowledge of the specificity of the enzyme and the thermodynamic constants of the molecules involved should allow prediction of the final equilibrium[21].

2.4 *Elements of specificity*

Specificity is one of the most general properties of enzymes; it implies that only certain molecules of those having a structure which could undergo the general reaction catalysed by the enzyme are in fact substrates for it. Substances with molecules that do not fit into quite narrow categories of size, shape, electric charge, etc., do not react with the enzyme. Specificity can be as narrow as that of urease which will accept urea and nothing else, and as broad as that of some peptidases and esterases which demand little more of their substrates than that they contain the peptide or ester linkage.

The specificity of glycosidases is twofold—towards the donor and towards the acceptor. Although the narrow donor (substrate) specificity is conventionally recognized in the names given to glycosidases — β-glucosidase, α-galactosidase, polygalacturonidase and so forth — it is not so generally realized that the number of substances capable of acting as acceptors to a given enzyme is often equally restricted. When the enzyme transfers a group to a sugar residue or chain of sugar residues as in the case of dextransucrase[22], Q-enzyme[16], D-en-

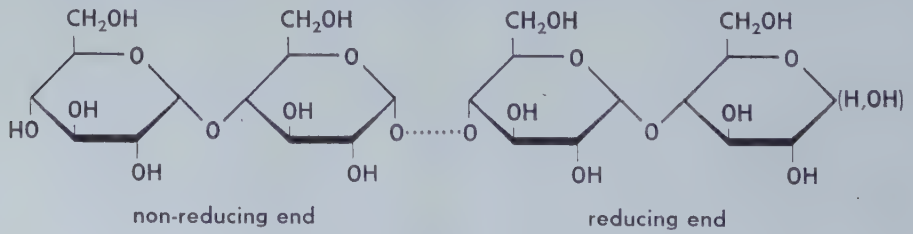


Figure 10

zyme[17], etc., it is not considered surprising that polysaccharides of quite specific structure are built up by transfer to only one or a few of the available hydroxyl groups. However Takano[23], using a series of glycosidases and simple hydroxyl compounds that were allowed to compete with each other, or with water, for the transferred glycosyl groups, has shown that each enzyme has a characteristic pattern of preferred acceptors.

2.5 *Specificity of exoglycosidases*

Since a polysaccharide chain consists of a series of sugar residues bound together by glycosidic linkages, the two ends of the chain are not the same. This is illustrated in Figure 10 for the poly-α-glucosidic chain of amylose (one of the polysaccharides present in starch). In the sugar residue at one end of the chain (non-reducing end) carbon atom 1 is bound in a glycosidic linkage, in the sugar at the other (reducing end) it is not so bound and the two configurations of the sugar can interconvert through the open-chain aldehyde form and the terminal sugar residue can thus exhibit all the properties of a free reducing sugar. A glycosidase removing a terminal non-reducing sugar from the chain is thus acting in the same way as a simple glycosidase breaking the glycosidic linkage between a single sugar residue and a simple aglycone, the whole of the rest of the polysaccharide chain now acting as aglycone. It is convenient to group all such enzymes together as exoglycosidases. In fact, as will be seen, many enzymes are capable both of splitting simple glycosides and of removing the terminal non-reducing sugar from polysaccharide chains.

One of the long-standing problems in the field is as follows: given a substrate for which a certain enzyme is specific, what are the elements in the structure of the substrate for which the enzyme is specific and what does the specificity consist of in terms of the structure of the enzyme protein? For exoglycosidases we may simplify this problem by arbitrarily dividing the substrate into a sugar moiety, an aglucone moiety and the configuration of the glycosidic linkage.

Not all of the substances carrying some or all of these structure elements are substrates for the enzyme, and the technique of "competitive inhibition" must be used. The generally accepted theory of enzyme action of Michaelis and Menten[24] pictures substrates as combining reversibly with the active centre of the enzyme. Substances which are sufficiently related in structure to substrates to combine reversibly with the enzyme without being subjected to enzymic reaction will impair the observed action of the enzyme when exposed to it simultaneously with a true substrate. Such action is known as "competitive inhibition". A competitive inhibitor can be diagnosed and the degree of its affinity for the enzyme measured by the methods first systematized by Lineweaver and Burk[25], by observing its effect on the kinetics of enzyme action in certain situations.

It appears that, in general, substances bearing a structural likeness to the sugar moiety or having a glycosidic linkage with the same configuration can act as competitive inhibitors, but substances resembling the aglycone can not. This relationship may of course be somewhat obscured when aglycone and sugar moieties are alike in structure. All three elements must be present for a compound to act as a substrate; the specificity for the sugar moiety usually becomes much narrower and a marked aglycone specificity often becomes apparent. Thus, to cite but one instance, the writer[26, 27], working with a β-glucosidase from the mould *Stachybotrys atra,* found it to be competitively inhibited by glucose and many related substances and by many β-glycosides, but not by aglycones or their analogues. However, only aryl β-glucosides would serve as substrates; other β-glycosides and alkyl β-glucosides were not hydrolysed. The only change possible in the molecule of an aryl β-glucoside which did not lead to loss of substrate activity was 6-substitution in the sugar ring, e.g. to give *p*-nitrophenyl 6-O-methyl or 6-O-toluenesulphonyl-β-D-glucoside.

In general, specificity toward the sugar moiety, either in competitive inhibitors or in substrates, is relatively easy to test since monosaccharides and oligosaccharides provide a series of natural analogues for each other which can be fairly readily modified by transformation into glycosides of different sorts or by effecting various substitutions on the sugar rings.

One of the most striking regularities emerging from this type of research is that many simple glycosidases are strongly inhibited by the corresponding aldonolactone[28]. The relationship of this substance to the corresponding sugar glycosides is shown in Figure 11 for the case of glucose.

The factors that lead to the enzymes having much higher affinity for these lactones than for

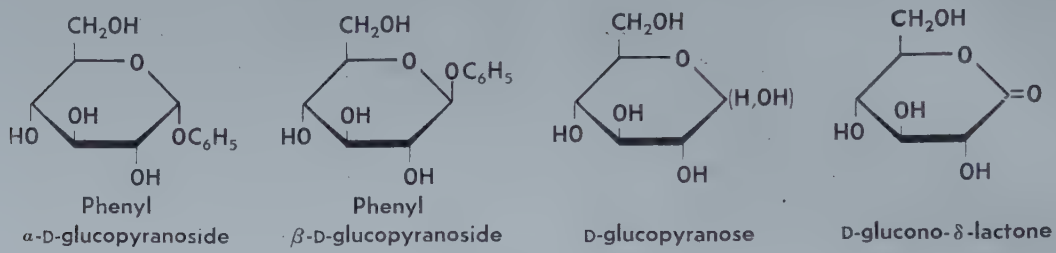


Figure 11

the corresponding sugars are at present obscure. The generalization is not true for all exoglycosidases. In the case of the *Stachybotris atra* β-glucosidases, the aryl β-glucosidase is strongly inhibited by ..-glucono-δ-lactone[26], the 'cellobiase' (an exoglycosidase which hydrolyses terminal β-glucosyl groups) is not[29]. Such differences in inhibition may be pointers to important differences in enzyme mechanism.

Alteration in the ring form of the sugar invariably produces a non-substrate, e.g. the β-galactosidase of *Escherichia coli* will hydrolyse phenyl β-D-galactopyranoside but not phenyl β-D-galactofuranoside[30], nor are there any reports of enzymes that will hydrolyse both α- and β-glycosides of the same sugar, however, such compounds will act as competitive inhibitors in some cases. Total inversion of the structure of the sugar is incompatible with enzyme activity. Thus the readily available D- and L-arabinosides are hydrolysed by unrelated enzyme systems. Inversions and eliminations of hydroxyl groups on the sugar ring to give glycosides of sugars other than the typical 'substrate' glycoside very often give competitive inhibitors. By studying what changes are possible without eliminating affinity for the enzyme we can often get some insight into the mechanism of attachment of the substrate to the enzyme. Thus the β-glucosidase of *S. atra*[26, 27] will combine with molecules where the hydroxyl groups on C2 have been inverted, substituted or eliminated and it may be concluded that this hydroxyl group plays no part in the attachment of substrate to this enzyme. But compounds of this sort are more rarely substrates, i.e. there are very few glycosidases of wide specificity so far as the sugar moiety is concerned. Perhaps the best-known examples of such enzymes are the "emulsins" of the seed-kernels of Rosaceous plants, classically of almonds. These preparations exhibit a wide variety of glycoside activities. Beibel[31] summarizes the confusing hypotheses about their nature which have swung back and forth between the extremes of "one substrate—one enzyme" and a single totipotent enzyme. It would appear at present that there is no proof of the existence in the emulsins of pure homogeneous enzyme proteins with action on more than one glycoside type and there is much suspicion that they may be non-existent [32, 34]. These remarks apply to all other parallel cases in the literature and the whole question must be regarded as unanswered.

The genuineness of absolute aglycone specificity in exoglycosidases is a vexed question. Examples range from the aryl β-glucosidase of *S. atra*[26] which will hydrolyse aryl β-glucosides and nothing else to the α-glucosidase of *Candida tropicalis*[14] which will remove glucosyl groups from phenyl α-glucoside, methyl α-glucoside, sucrose, maltose and starch. Such observations are perhaps to be explained in terms of relative rather than absolute specificities, i.e. all substrates are hydrolysed at much the same rate by the *C. tropicalis* enzyme but alkyl β-glucosides are hydrolysed so much more slowly than aryl-glucosides by the *S. atra* enzyme that the rate is experimentally zero for attainable conditions. In general, opinion has

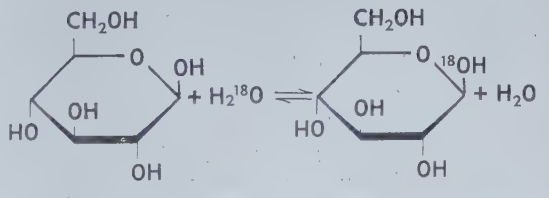
followed Pigman[36] who considers aglycone specificity purely relative. But there seems to be no operational difference between absolute specificity and extreme relative specificity. Even in cases where it has been claimed that an organism produces a series of enzymes of different absolute specificity, there has rarely been any proof that the specificities are more than relative, e.g. for the yeast α-glucosidases of Hestrin and Lindgren[37]. The pattern of activity in which an organism produces a series of enzymes differing only in relative specificities towards a group of substrates with the same sugar moiety but different aglycones seems, in fact, very common[33, 38].

Where an absolute aglycone specificity appears to exist, it is often probable that this observation means no more than that the kinetics of the reaction are such that it goes overwhelmingly in one direction. Thus levansucrose, which builds up polymers by transferring fructosyl groups from sucrose to polyfructose chains as acceptors, has been supposed to be irreversible in its actions, i.e. not to be capable of removing fructosyl groups from the end of the chain and transferring them to glucose. However, Péaud-Lenoël[39] has shown this to be merely a consequence of the thermodynamics of the reaction; by altering the conditions he could demonstrate the back-transfer reaction.

The question of reversibility becomes especially interesting when water is the acceptor and many glycosidases will in fact bring about the synthesis of glycosides from aglycone and sugar when these 'reaction products' are present in high concentrations. The use of almond emulsin for the synthetic preparation of β-glucosides by the reverse reaction is particularly well known [40, 41]. Whether this reaction is to be thought of as a simple reversal of hydrolysis GlyOH + ROH $\rightleftharpoons$ GlyOR + HOH depends on the view taken about the mechanism of enzyme reaction and there are reasons [20, 39] why the simplest explanation is possibly too simple. Koshland[4] has shown that β-glucosidase will catalyse the reaction (Fig. 11a), with water acting as both donor and acceptor.

Much of the work on aglycone specificity has been carried out on aryl glycosides, largely on aryl β-glucosides and β-galactosides which are comparatively easy and cheap to prepare. The effect of practically every possible variation in the substitution of the benzene ring has been investigated; similar systematic investigations have been carried out with alkyl β-glycosides although here the possible range of variation is narrower. The differing effect of *ortho*-substitution in phenyl β-glucoside on various enzymes finally destroyed the idea of a single "β-glucosidase" of constant properties from all sources. Thus O-cresyl β-glucoside is much more readily split than phenyl β-glucoside by β-glucosidases from higher plants[44, 45] but much less readily by fungal β-glucosidases. Further work and the growth of ideas lead inevitably to the conclusion that glycosidases of any type from various sources can not be expected to have similar properties other than the power of hydrolysing the substrate that first drew attention to them.

One of the broadest generalizations about specificity has been attempted by Japanese workers. They divide the glycosidases into *taka* and *emulsin* types[47, 49]. *Emulsin* β-galactosidase, to take an instance, is inhibited by aryl β-glucosides, glucose and gluconate ion and hydrolyses *o*-cresyl β-galactoside more rapidly than phenyl β-galactoside. *Taka* β-galactosidase is inhibited by aryl β-galactosides (other than the substrate), galactose and the galactonate ion and hydrolyses phenyl β-galactoside more rapidly than O-cresyl β-galactoside. But even for galactosidases from a group such as the fungi, these generalizations can be shown not to hold completely[50] and there is now fairly general agreement with the view of Miwa[51] that enzymes answering to the description of taka and emulsin types are merely extremes of a range.



β-D-glucopyranose

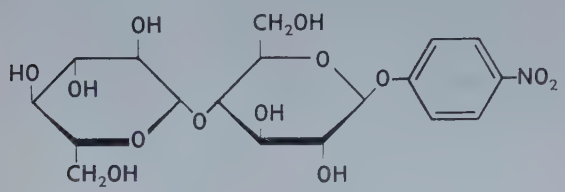
Figure 11*a*

2.6 *Specificity of endoglycosidases*

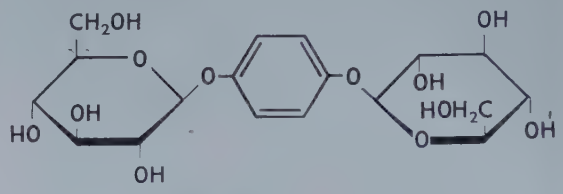
One generalization that seems to be true for enzymic attack on polysaccharide chains is that in every case there seem to be two types

of enzymes—those attacking endwise from the non-reducing end and those cleaving internal glycosidic linkages. Irrespective of whether any difference in enzyme mechanisms separates these two classes, it is certain that they are normally separated by profound differences in the size of the optimal substrate. But even these differences are not absolute. Thus the cellulase of *Myrothecium verrucaria* oligosaccharides and those that require large ones. An outstanding case of the latter is the endoxylanase of Sorenson[55] which appears to require a minimum chain length of about twenty residues.

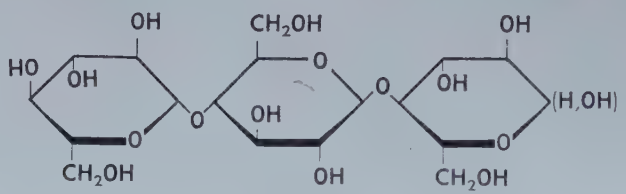
The specificity of endoglycosidases is one of the experimentally most difficult subjects in glycosidase chemistry and for this reason results are scattered and difficult to summarize.



p-nitrophenyl β-cellobioside



hydroquinone bis β-glucoside



cellotriose

Figure 12

[52] will hydrolyse cellobiose, i.e. β-glucosyl-(1→4) glucose, thus in the limit attacking the same substrate to give the same products as an exoglycosidase, the 'cellobiase' of *Stachybotrys atra,* even though the action of the two enzymes on long β-glucosyl-(1→4) chains may be quite different. The cellulase of *Stachybotrys atra* will not act on any substrate smaller than cellotriose[53], i.e. β-glucosyl–(1→4)-β-glucosyl– (1→4)glucose, and a similar minimum of three glycosyl units is found for many α-amylases. Yeast polygalacturonase[54] needs a sequence of four galacturonic acid units for effective action, although it will act very slowly on a sequence of three, and a similar indeterminacy about the size of the minimum oligosaccharide effective as a substrate is common in the literature. Whether the penultimate malto-oligosaccharide is hydrolysed by a given α-amylase not at all or merely very slowly has led to much controversy. This indeterminacy makes it difficult to give a fixed figure for minimum substrate chain length with many endoglycosidases, and it is often only possible to divide them into those that will act on small

Several methods of investigation have been pursued in appropriate cases. Where it is found that a certain oligosaccharide is a substrate for an enzyme it is possible to modify it in various ways to see how these affect the activity of the enzyme. It is difficult to make totally synthetic analogues of oligosaccharides, but one method, successful in some cases, is to put the terminal reducing sugar of the oligosaccharide that contains one less glucoside residue into glycosidic linkage. Thus the cellulase of *Irpex lacteus*[35] will hydrolyse both cellotriose and *p*-nitrophenyl β-cellobioside but not alkyl β-cellobiosides, cellobiose, or hydroquinone bis β-glucoside (see Figure 12). We can therefore conclude that the enzyme requires at least two consecutive β-glucosidic linkages, the first being between two glucose residues (the 'tail' of the molecule), the second being to a 'head' aglycone for which there are wide but not indefinite specificity requirements. Given this arrangement, Nisizawa and Hashimoto's result shows that some hydrolysis of the 'tail' β-glucosidic linkage is possible as well as the predominant hydrolysis of the

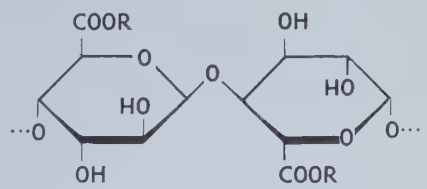
'head' linkage. *Stachybotrys atra* cellulose on the other hand which seems to hydrolyse cellotriose exclusively at the 'head' linkage has a much narrower aglycone specificity and will not hydrolyse *p*-nitrophenyl β-cellobioside[53]. How much narrower this specificity may be is not known, since suitable test substrates, e.g. trisaccharides with other sugars replacing glucose in the 'head' position have never been synthesized. The β-glucosidase of *Irpex lacteus* on the other hand acts on *p*-nitrophenyl β-cellobioside principally as an exoglycosidase, i.e. to give glucose and *p*-nitrophenyl β-glucoside, but shows a little endoglycosidase activity to give cellobiose and *p*-nitrophenol. A gradation between true exoglycosidase and true endoglycosidase activity can thus be shown with the enzymes of *Stachybotrys atra* and *Irpex lacteus* as demonstrated in Table 1. It should be added that this attractively simple picture depends on the validity of Nisizawa's belief that his enzymes are not cross-contaminated.

Chemical modification of known substrates has been largely confined to reduction or oxidation of the terminal reducing residue, although other techniques such as selective substitution in certain positions may be possible. The galacturonide chains of the oligosaccharides derived from pectic acid with their attractive additional possibilities of esterification of the carboxyl groups have given the most productive results by this method, although it has also been applied to the study of the action of α-amylases on the poly-α-glucosidic chains of the malto-oligosaccharides derived from starch.

Table 1 *Enzymes of* I. lacteus *and* S. atra

Enzyme	Action on *p*-nitrophenyl β-glucoside	Action on *p*-nitrophenyl β-cellobioside	Interpretation
β-Glucosidase of *S. atra*	→ glucose + *p*-nitrophenol	none	Aglycone–specific. 4-Substitution in β-glucoside gives a non-substrate
Cellobiase of *S. atra*	→ glucose + *p*-nitrophenol	→ glucose + *p*-nitrophenyl β-glucoside	Aglycone–unspecific. 4-Substitution the β-glucoside gives a non-substrate
β-Glucosidase of *I. lacteus*	→ glucose + *p*-nitrophenol	→ glucose + *p*-nitrophenyl β-glucoside (major pathway) → cellobiose + *p*-nitrophenyl (minor pathway)	Aglycone–unspecific. 4-Substitution in the β-glucoside gives partial inhibition
Cellulase of *I. lacteus*	no action	→ glucose + *p*-nitrophenyl β-glucoside (minor pathway) → cellobiose + *p*-nitrophenyl (major pathway)	Aglycone–unspecific. 4-β-Glucosyl substitution in a β-glucoside almost essential
Cellulase of *S. atra*	no action	no action	Aglycone–specific. 4-β-Glucosyl substitution in the β-glucoside quite essential

Pectic acid is poly-(1→4)-α-D-galacturonic acid (Figure 13) and occurs in nature as its methyl ester, pectin. Polygalacturonases can act on pectin provided that it is sufficiently demethylated, the evidence indicating that for



pectic acid when R=H

pectin when R=Me

Figure 13

yeast polygalacturonase both galacturonic acid residues about the susceptible linkage must be demethylated while for fungal polygalacturonases only the 'tail' residue need be demethylated. Polygalacturonases from different sources show a great variation in specificities and some of the information is summarized in Table 2.

Table 2 Reactions of polygalacturonases

Substrate	Yeast polygalacturonase[54]	Tomato polygalacturonase[58]	Exogalacturonidases from fungi[57, 58]
GaA_1*–GaA_2–GaA_3–GaA_4	GaA_1–GaA_2–GaA_3 + GaA_4	{ GaA_1–GaA_2–GaA_3 + GaA_4 { GaA_1–GaA_2 + GaA_3–GaA_4[a]	
GaA_1*–GaA_2–GaA_3–GaA_{ox}†	GaA_1–GaA_2 + GaA_3–GaA_{ox}[b]	GaA_1–GaA_2 + GaA_3–GaA_{ox}[b]	
GaA_1–GaA_2–GaA_3–GaA_{red}‡	not attacked	GaA_1–GaA_2 + GaA_3–GaA_{red}[b]	
GaA_1–GaA_2–GaA_3	GaA_1–GaA_2 + GaA_3[b]	GaA_1–GaA_2 + GaA_3[b]	GaA_1 + Ga_2–GaA_3
GaA_1–GaA_2–GaA_{ox}	not attacked	GaA_1 + GaA_2–GaA_{ox}[c]	
GaA_1–GaA_2–GaA_{red}	not attacked	GaA_1 + GaA_2–GaA_{red}[b]	
GaA_1–GaA_2	not attacked	GaA + GaA[b]	GaA + GaA
GaA_4–GaA_{ox}	not attacked	not attacked	GaA + GaA_{ox}
GaA–GaA_{red}	not attacked	not attacked	GaA + GaA_{red}

* GaA = D-galacturonic acid
† GaA_{ox} = oxidized D-galacturonic acid (mucolactone)
‡ GaA_{red} = reduced D-galacturonic acid (L-galactonolactone)
a = minor pathway
b = slow
c = very slow

The facts are complex and no simple theory can explain all of them in detail, but it is clear that yeast polygalacturonase is aglycone specific and requires a 'tail' of at least two or three residues. Tomato polygalacturonase is aglycone specific, and although it can be active with a tail of one unit, at least three are preferable. The fungal enzyme is a typical exoglycosidase with no aglycone specificity.

The requirement for a tail of 1,2 . . . *x* glycosidic linkages seems to be typical of endo-glycosidases. Although the endo-enzymes of Table 2 may split those linkages in oligogalacturonides fastest which are furthest from the nonreducing end, this does not indicate that enzyme action is enhanced by proximity of the linkage split to the reducing end, i.e., to small size of the aglycone. The enzymes split the internal linkages in long-chain polygalacturanides even faster. Beyond the fact that both galacturonic acid and galacturonic acid substituted on C1 are suitable aglycones and L-galactonolactone and mucolactone are not, nothing can be said for the polygalacturonidases about aglycone specificity and, except for the inhibiting effect of esterification, nothing about the effect of alteration in the 'tail'. However, since many other natural polysaccharides are not regular chains but have irregular linkages or branches at various points, the study of the action of enzymes on these polysaccharides often throws some light on the effect of alterations and substitutions on the chain on the action of endo-enzymes.

One of the most straightforward of such studies is that of Kooimans[59] on the action of *Myrothecium verrucaria* cellulase on the "amyloid" of *Tamarindus indica*. The "amyloid" consists essentially of a cellulose chain with glucose residues substituted by xylose residues, some of these latter again being substituted by galactose residues. The action of the cellulose leads to oligosaccharides containing chains of four glucose residues, the first three from the non-reducing end being substituted by xylose and the fourth unsubstituted. The amyloid thus has a general structure of the type shown in Figure 14, with the bonds indicated being split by the enzyme. *M. verrucaria* cellulase thus requires that the glucose nearest to the cleaved linkage in the tail be unsubstituted, but will accept a substituted polyglucose chain as aglycone.

This approach has been applied most often to the polysaccharides of starch[60, 61]. β-Amylase removes α-glucosidic units two at a time

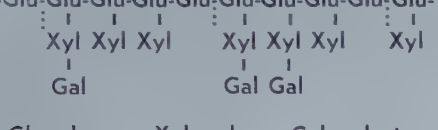


Figure 14

from the non-reducing end of a poly-(1→4)-α-glucosidic chain, and its action on the linear amylose from starch has shown in many cases the presence of anomalous linkages beyond which the enzyme can not act[62]. Allowed to act on amylopectin, which contains branches beginning with an α-(6→1) linkage as well as poly-α-(1→4) chains, β-amylase can apparently "see" the branches two or three residues ahead and its action stops. This demonstration that a length of the chain on the 'head' side of the cleaved linkage is involved in β-amylase action is important in theories of enzyme mechanism. α-Amylase on the other hand can cleave linkages up to the one before the glucose residue on which the branch occurs but needs at least one or two (the number depending on the nature of the substrate chain) unsubstituted glucoses in the 'tail' before it can cleave an-

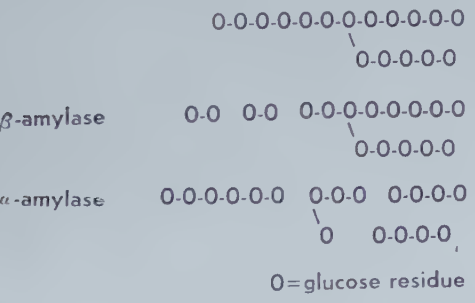


Figure 15

other linkage. These results suggest that α-amylase will accept an α-(1→6) linked glucose as the second residue in the tail but not an α-(1→4) linked substituted glucose (see Figure 15).

The final but most laborious method that can be used is the paper chromatographic study of the mixture of oligosaccharides present at any time during the attack on a longer chain oligosaccharide by the enzyme. By judicious statistical interpretation of the results it is possible to assess the relative susceptibility of different linkages to the action of the amylase. If substrates can be synthesized, usually enzymatically, such that indicator sugar residues containing ^{14}C atoms at known places in the chain, the analysis can be much refined. For salivary α-amylase the pattern given in Figure 16 is found[59]. This suggests that the α-amylase

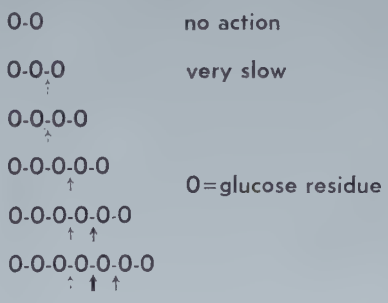


Figure 16

has some action on every linkage except the first but that the absolute and relative amount of attack on any bond is a function of the molecule as a whole.

2.7 *Acceptor specificity*

Some of the most painstaking work on acceptor specificity of glycosidases has been done by Miwa's group in Japan[23, 63–67]. These results can not be easily summarized in detail but Table 3, taken from Suzuki[65], illustrates their importance for any theory of acceptor specificity. Except that concentration and other variables were constant throughout, the exact experimental conditions are unimportant. Besides the quantitative data in the table, Suzuki was able to check his main conclusions by paper chromatography. The conclusions that emerge are the following.

(1) Each enzyme has its own particular pattern of specificity: there is no such thing as a general "β-glucosidase". Nevertheless the enzyme appears to be identical in different portions of the peach plant.

(2) Acceptor specificity is quite as basic a property of these enzymes as donor specificity. We should expect different β-glucosidases to react with the components of mixtures of

substrates ('donors') in characteristic ratios which would depend on the different relative affinities of the substrates for the different enzymes. But Suzuki further shows that β-

(4) In many cases a sugar appears to inhibit transfer of the β-glucosyl group to the glucoside acting as acceptor. This can only be simply explained if the sugar acts by being

Table 3 *Degree of transglucosylation from* p-*nitrophenyl* β-D-*glucoside to alcohols and sugars by β-glucosidases from different sources*

Enzyme source		β-Glucosyl transfer, %, to					
		None*	Methanol	Glycerol	Glucose	Fructose	Xylose
Cycas revoluta	A	7	41	23	±	±	+
(cycad; seeds)	B	0	34	16	—	—	—
Ginkgo biloba	A	6	20	31	0	0	9
(gingko; leaves)	B	0	14	25	—	—	±
Prunus Armeniaca	A	0	4	11	0	0	0
(apricot; seeds)	B	0	4	11	0	0	0
Prunus Persica	A	0	5	15	0	0	0
(peach; seeds)	B	0	5	15	0	0	0
P. Persica	A	0	8	15	0	0	0
(leaves)	B	0	8	15	0	0	0
Gentiana scabra	A	13	20	39	11	6	7
(gentian; roots)	B	0	7	26	—	—	—
Datura Tatula	A	20	31	50	17	14	16
(leaves)	B	0	11	30	—	—	—
Plantago major	A	14	24	41	22	16	19
(plantain; leaves)	B	0	10	27	8	±	+
Oryza sativa	A	40	48	67	41	27	33
(rice-plant; ears)	B	0	8	27	0	—	—

A Total transfer (sum of β-glucosyl group transferred both to donor acting as its own acceptor and to added acceptor)
B Transfer to added acceptor
* Transfer to donor molecule acting as its own acceptor
± Degree of transfer is exceedingly small
\+ Degree of transfer is small, but evident
— Degree of transfer is lower than that in the absence of the added sugars; it seems that the transglucosylation to substrate molecule is inhibited by the sugar added

glucosidases react with a mixture of possible acceptors (water, alcohol and the glucoside as its own acceptor) in characteristic patterns. Takano[23], using a long list of acceptors but fewer enzymes, has shown in finer detail that no two acceptors have identical efficiency as competitors of water for a given glycosidase and that the relative order of these efficiencies, as well as their absolute amount, changes with the change from one acceptor to another.

(3) In general the sugars could not act as acceptors with any efficiency, but for all the β-glucosidases other than those of the *Prunus* species the glucoside could act as its own acceptor giving *p*-nitrophenyl β-cellobioside and gentiobioside as primary products. Thus, for some of the enzymes there is a wide relative specificity difference between the sugar and the glucoside as almost to appear absolute; there is a much smaller relative specificity difference between simple acceptors.

attached to the molecule in the "acceptor" position, thus blocking the approach of a competent acceptor. Suzuki's table is prepared on the assumption that self-transfer in the absence of acceptor can be deducted from total transfer in its presence to give transfer to the acceptor. Competition at the acceptor site is inferred only from the reduced total transfer with non-acceptors. But in fact the presence of a further alternative acceptor besides water and the glucoside must have the same result of altering the amount of transfer to the glucoside as the presence of a competitive non-acceptor; Suzuki's values in his "B" row are therefore lower limits to the amount of transfer rather than low values. For simpler systems (no self-transfer), Takano has shown that methanol and water are competitors for the β-glucosidase of the apricot; as the methanol concentration rises the total rate of en-

zyme reaction falls, but an increasing proportion of the transfer is to methanol. For the β-glucosidase of *Stachybotrys atra* acting on the same substrate (*p*-nitrophenyl β-glucoside)[27], rising concentrations of glycerol speed up the reaction and transfer an increasing proportion of the β-glucosyl residue to glycerol; rising concentrations of methanol decrease the reaction rate but the transfer is increasingly to methanol.

The simple explanation of these results is competition of substances for the acceptor site, the affinity of this site for these substances being quite independent of their efficiency as acceptors when bound to it. Such competition at the acceptor site should give rise to typical kinetic behaviour—the obscurely named "anti-competitive inhibition"[20], and there is sufficient independent evidence of acceptor competition to argue back cautiously from observed anti-competitive inhibition when it is observed in glycosidases to acceptor competition in selected cases[27].

Not enough is known of the details of acceptor specificity to make it safe to generalize, as is shown by the results of Hash and King[68] on another fungal β-glucosidase, that of *Myrothecium verrucaria*. Here most of the results can be explained in terms of the hypothesis advanced above. Yet the amount of transfer to water is sometimes increased by the presence of a second acceptor; also some non-acceptors increase the rate of enzyme action, where they should only be capable of decreasing it. The hypothesis of acceptor competition can be saved by supposing that alcohols can exert (by unknown means) a general activating effect on the *M. verrucaria* enzyme. But this is only an *ad hoc* explanation, and there is not sufficient information on the acceptor specificity of glycosidases to systematize the few observed cases. Nevertheless, the main facts of acceptor specificity—wide specificity for simple molecules, including water, narrower specificity for sugars, and acceptor competition—are not in doubt. Where sugars are the acceptors some enzymes can transfer to any available hydroxyl on the sugar[69], some are more limited in their choice[70] and others require the sugar to be involved in another glycosidic linkage[65], to be the end of a glycosidic chain[19] or to be an internal residue in a chain[17]. Almost every possibility that is to be found in donor specificity can be paralleled in acceptor specificity.

Any theories of the mechanism of glycosidases therefore must take into consideration the fact that the acceptor and donor must be regarded as co-substrates of the enzyme. The present lack of knowledge of the kinetics of enzyme action with the acceptor regarded as a substrate, means that we must either formulate mechanisms with detailed information about only one half of the reaction or take the risk of generalizing from the few, and perhaps totally unrepresentative, examples of acceptor kinetics that have been presented.

3 MECHANISMS

The preceding section has given a brief and generalized survey of the kinds of observational data that must be assessed in deriving mechanisms for glycosidases and this section will consider some of the mechanisms that have been advanced.

3.1 *The fission of glycosidic linkage*

The chemical hydrolysis of the glycosidic linkage can take place under two different sets of conditions and by two separate mechanisms. The glycosidic linkage is readily broken by acid hydrolysis; it is highly resistant to alkaline hydrolysis to the point where simple alkyl glycosides remain unattacked unless extremes of temperature and alkali concentration are used. However, in the case of aryl glycosides the linkage becomes more and more labile to alkali as the aglycone assumes the character of an "electron sink". Nitrophenyl glycosides are about equally labile to acid and alkali; dinitrophenyl glycosides are unstable in neutral solution.

The mechanism of the alkaline hydrolysis of glycosides is conceptually simple, although the course of the purely chemical reaction is much more complex than the simple theory suggests. It is believed that the displacing reagent is a negative ion (a "nucleophilic' reagent) and the reaction takes place by "back-side displacement" according to the S_N2 mechanism (Figure 17).

It will be noticed that this mechanism involves Walden inversion of the configuration

about the glycosidic carbon atom. In the actual reaction the OH groups on C2 and C6 (in a

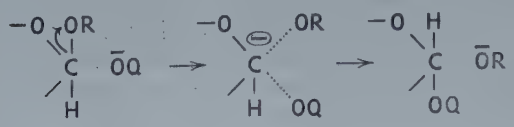


Figure 17

hexoside) become involved, the C2 group normally having to be free for easy alkaline hydrolysis. But it should be remembered that the direction of free energy change favours the hydrolysis and its slowness and tendency to take by-paths is a reflection of the very high potential energy barriers to be overcome. There is no reason to suppose that an enzyme could not catalyse the simple reaction as shown.

The acid hydrolysis is believed to proceed by way of protonation of the glycosidic oxygen followed by solvolysis of the carbonium ion so formed (Figure 18). In this mechanism the configuration about the glycosidic carbon would be retained in the simple case, although mechanisms of solvolysis could be imagined that would invert it. The mechanisms for both acid and alkaline hydrolysis which involve cleavage between the glycosidic carbon and the

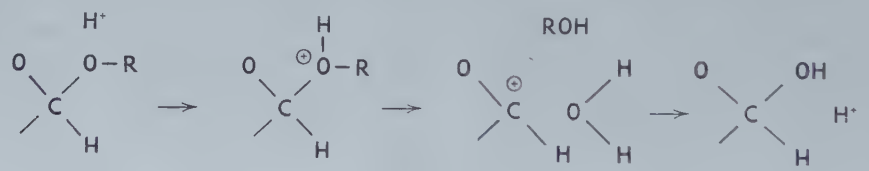


Figure 18

oxygen, are consistent with the results of isotope studies for both chemical and enzymic hydrolysis.

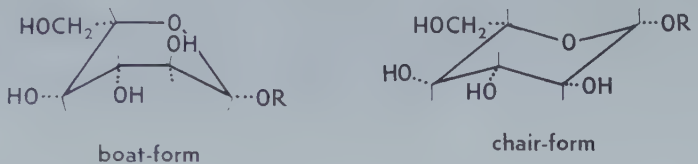
Koshland[3] proposes that the function of those glycosidases that invert the configuration of the reaction product is to promote "backside" attack on the glycosidic carbon atom by a nucleophilic water molecule. This "promotion" is of course to be understood in terms of electronic attractions mediated by the way in which the substrate is attached to the active site of the enzyme protein. Koshland ascribes retention of configuration to double inversion; the first nucleophilic reagent is part of the active enzyme site. The glycosyl residue is bound to the enzyme in the reversed configuration; this enzyme-glycosyl compound then reacts with the second nucleophilic reagent (acceptor) to give the transfer product with reacquisition of the original configuration.

It is a feature of Koshland's hypothesis that the bond broken is that between the moiety of the glycoside for which the enzyme is specific, and the glycosidic oxygen. A single-stage transfer with retention of configuration could only occur if the enzyme is specific for the aglycone, and the bond between aglycone and oxygen is the one that is broken. Such a mechanism may just be possible for glycosyl transfer from uridine diphosphate (UDP) sugars, since it is at present uncertain in many cases whether the enzymes involved are specific for UDP or sugar[15], i.e., whether there is one UDP-glycosyl transferase or many. Some UDP-glycosyl transferases however are quite definitely sugar-specific and single stage transfer with retention of configuration by the Koshland mechanism would be impossible. Such transfer is also incompatible with the known facts about the glycosidases. For those UDP-glycosyl transferases that act with inversion of configuration, single stage transfer by the mechanism postulated by Koshland for β-amylase would be possible.

The hypothesis of Mayer and Larner[7] depends on the fact that the six-membered pyranose ring is not in fact planar as represented in the Haworth formula. It is puckered out of a plane and a large number of different "conformational" structures are possible for the heterocyclic ring. For an α-glucoside, two of these forms, the "boat" and "chair" conformations are given in Figure 19. The equatorial (e) bonds are drawn as dotted

lines and lie in a single plane; the axial (*a*) bonds lie perpendicular to this plane and are shown as full lines. Although the conformation adopted by a given sugar unit, either alone or as part of a glycoside or polysaccharide, is known to be stable in the solid state and even under specified conditions in solution[71–73], enzymologists have been cautious of giving explanations based on conformation since all the various conformations are interconvertible.

Figure 19



Even if the conformation of the substrate in solution were known with certainty, it would remain uncertain whether the substrate was bound to the substrate in the same conformation. Indeed, one of the essential functions of a glycosidase may be to make a reaction possible by altering the conformation of the substrate, since many groups differ markedly in reactivity according to whether they are in axial or equatorial positions.

The conformation of the sugar units of polysaccharides may well be fixed since there is often a complex secondary and tertiary structure over and above the primary valence bond structure. Mayer and Larner accept the evidence of Reeves[74] that the glucosyl units in amylose are essentially in the boat form, while free reducing sugar units tend to be in a chair form. Figure 20 is taken from their paper, since it is almost impossible to give a simple verbal description of their hypothesis. In essence, the surface features of the two enzymes necessitate that the ionic intermediate assumes different conformations. Solvolysis by the S_N1 mechanism, in which the direction of attack determines the configuration of the new bond formed, gives rise to different final configurations in the product. Although this theory explains simple hydrolysis readily, it is uncertain how far it is applicable to acceptors other than water.

Before discussing these two theories further, enzyme-substrate-acceptor relationships must be considered.

3.2 *The role of the acceptor*

There are two ways of picturing the role of the acceptor in glycosidase action. In the first, transfer is visualized as a two-stage process:

$$EOH + ROGly \rightarrow EOGly + ROH$$

(E = enzyme molecule)

$$EOGly + R'OH \rightarrow EOH + R'OGly.$$

In the second, enzyme, substrate and acceptor are believed to be bound in a single complex and it is only after the formation of this ternary complex that any decomposition to give the products of the enzyme reaction can take place. The first picture is most compatible with the Koshland mechanism for enzyme action, the second with the Mayer-Larner mechanism. The attack on the carbonium ion intermediate postulated by Mayer and Larner would take place either by an acceptor molecule bound very specifically to the enzyme surface, or in most other cases much less specifically, by water or other small molecules from the solvent, thus neatly accounting for the facts of acceptor specificity. Obligate hydrolases would have no provision for specific binding of an acceptor. The writer[20] has tried to combine the ternary complex picture with Koshland's theory by proposing that the first transfer to the nucleophilic centre on the enzyme can not take place until the acceptor is bound in position. There is no current evidence that this is an impossible mechanism, but it is obviously much less simple than the straightforward Mayer-Larner interpretation.

One important qualitative prediction can distinguish the two hypotheses about acceptor action. On the two-stage hypothesis, the nature of the acceptor can affect the ratio of transferase to hydrolase action whereas the nature of the donor can not. This is because all donors give rise to an identical EOGly intermediate. On the ternary complex hypothesis, the nature

of the donor can not only control this ratio by affecting the amounts of competing enzyme-donor-acceptor complexes found and their rate

variety of donors (phenyl α-glucoside, methyl-α-glucoside, sucrose, maltose) to fructose. Both the types and the relative amounts of the posi-

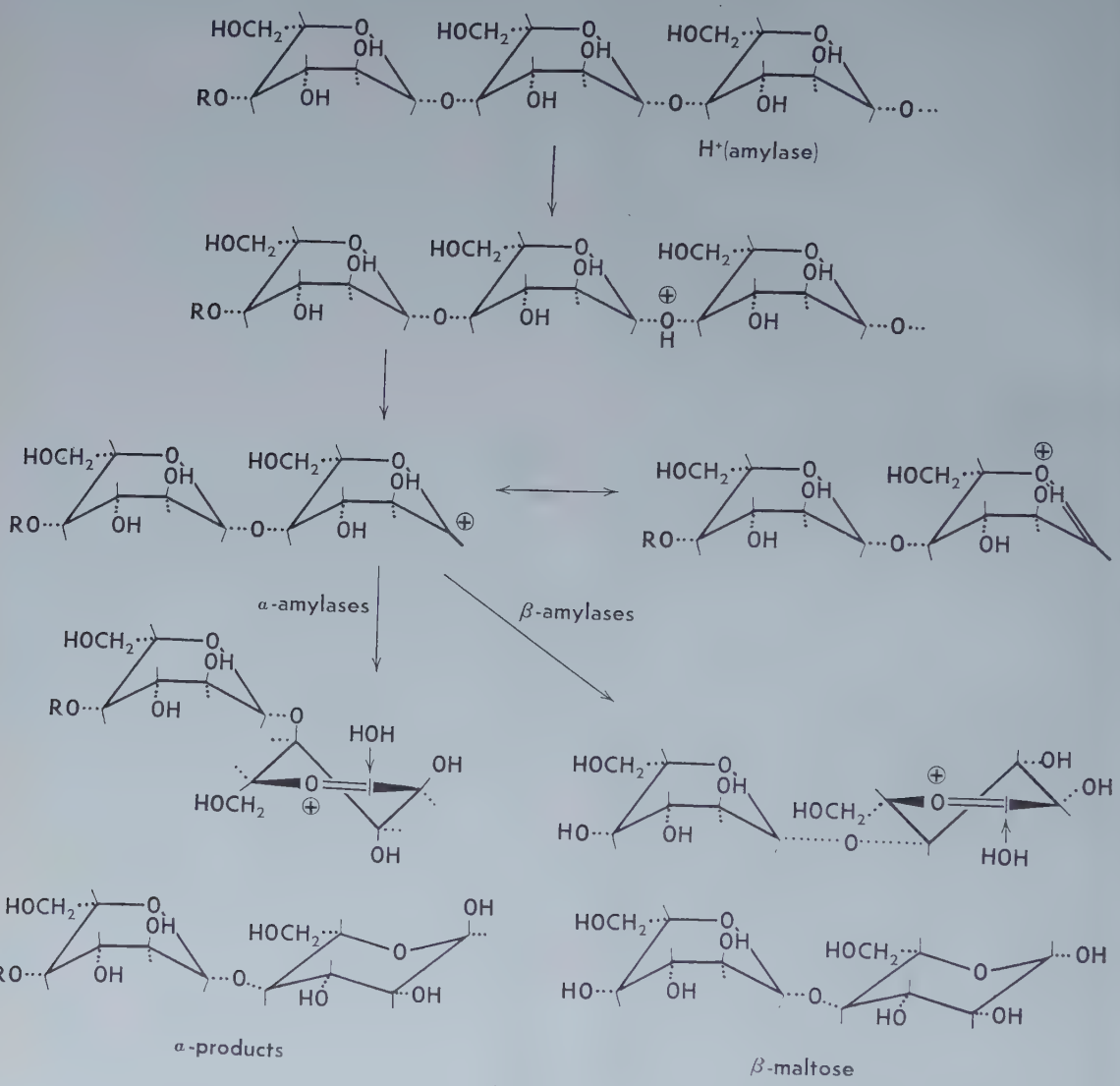


Figure 20

of decomposition, but it can also control the proportion of transfer to various sites on a complex acceptor (e.g. to various hydroxyl groups on a sugar) by affecting the probabilities of the various ways in which the ternary complex can break down.

A crucial experiment has been performed by Miwa *et al.*[69]. An α-glucosidase from bakers' yeast transferred an α-glucosyl residue from a

tional isomers were found to be dependent on the donor. If it is assumed that their enzyme preparation was homogeneous, the observations can only be explained on the ternary complex hypothesis. However, this assumption may be unjustified; for instance, Sawai[14] believed that his maltose from *Candida tropicalis* gave different ratios of transfer product to hydrolysis product depending on the donor but

was later forced to withdraw the implications of this observation because he found that his preparation contained at least two enzymes[75]. A careful search for phenomena of this type using enzyme preparations known unequivocally to contain only a single species is needed before the evidence can be finally assessed. This is one of the most important unanswered questions in the biochemistry of the glycosidases.

Convincing evidence that transferring enzymes other than glycosidases can act through a ternary complex has been provided for the enzymes that catalyse the formation of uridine diphosphate sugars from uridine triphosphate and sugar phosphates[76, 79]. The overall reaction is

uridine-PP*P* + P-sugar ⇌ uridineP-P-sugar + P*P*

(P = phosphoric acid residue, P* = radioactive P).

Using labelled phosphorus in the positions indicated, it can be shown that there is no exchange of radioactivity between uridine triphosphate and pyrophosphate except in the presence of sugar 1-phosphate. In other words, no free uridine phosphate enzyme complex is formed, and the reaction must proceed through a ternary enzyme-UTP-sugar1-phosphate complex. There is also kinetic evidence that many glycosidases act through the ternary complexes whose existence have been demonstrated by these experiments.

Neely and Thompson[78] have now shown by kinetic analysis that dextransucrase must act through a ternary complex. This enzyme transfers an α-glucosyl from sucrose to suitable carbohydrate acceptors but not to water, i.e. it is not a hydrolase, and its kinetics have been known for some time to defy explanation by conventional concepts[21]. Using sucrose as both donor and acceptor, they showed a sharp maximum in the rate of reaction as the enzyme concentration increased, other conditions being kept constant. This they interpret by supposing sucrose to be bound at both donor and acceptor sites; as the enzyme concentration rises the proportion of enzyme sites occupied simultaneously by donor-acceptor pairs must fall and with it the rate of reaction. A reaction between substrate and unbound acceptor could not give the observed kinetics[79].

Neely and Thompson point out that a maximum such as they have encountered is impossible where the solvent (water) is an alternative acceptor and the actual demonstration of ternary complex formation is very difficult in such a case. However, the writer[27] has pointed out that the kinetics of "anticompetitive" inhibition of the β-glucosidase of *S. atra*, i.e. inhibition by blockage of the acceptor site, can only be simply explained in terms of ternary complex formation. In this case and others it would, however, be possible to formulate other more complex mechanisms to fit the observed facts.

It may be concluded that the ternary complex mechanism is almost certain for a few cases, probable for some others and may be extended from these to the rest mainly on the grounds of simplicity. If it is accepted, the probability of the Mayer-Larner hypothesis is also increased at the same time, but the greatest need at the moment is for more experimental facts.

3.3 *Donor-acceptor relationships*

Suzuki[66] found β-glucosidases from higher plants could in general transfer a β-glycosyl residue to the substrate β-glucoside but not to glucose or other sugars, which, however, were able to block transfer. Hofstee[80] found non-competitive inhibition, probably due to blocking of the acceptor site, to be more significant than the expected competitive inhibition when competing β-glucosides are acted on by almond emulsin. The writer[27] found that sugars and the substrate β-glucoside itself block transfer from the *S. atra* β-glucosidase, but that a 6-O-methyl β-glucoside was an efficient acceptor for self transfer. Neely and Thompson remain noncommittal on the question of whether the "acceptor" and "donor" centres in dextransucrase are one and the same. In general it appears that very similar molecules are bound at both acceptor and donor centres and in both cases the requirements for acting as an acceptor or donor once bound are not the same as for the original binding.

It is difficult to escape from the conclusion that the neighbouring donor and acceptor centres, with their affinities for similar molecules, represent exactly the same type of centres as those that must be postulated as combining with the sugar residue on both sides

of the glycosidic linkage attacked by endoglycosidases. The fission and synthesis of glycosidic linkages then take place by the same mechanism with the added proviso that steric considerations may prevent a residue, that can be bound satisfactorily as the aglycone portion of a glycoside, being bound in the correct position to take place in the reactions leading to its resynthesis. Such proposals essentially revive the old hypothesis that the prosthetic group of glycosidases is carbohydrate in nature[81] without postulating an integral union between carbohydrate and enzyme.

The postulate that fission and synthesis are two aspects of a single mechanism raises no difficulties on the hypothesis of transfer by successive reactions; however, the ternary complex hypothesis leads to difficulty in the case of transfer by an endoglycosidase to a sugar acceptor. Either there must be three closely associated sites, two to accommodate glycosyl and aglycone moiety, one to accommodate acceptor, or else the enzyme can not bind, and therefore must be highly unspecific for, the aglycone. The first suggestion can not be disproved *a priori* but seems too complex to be a welcome first hypothesis. The second suggestion implies that non-transferring endoglycosidases are aglycone specific and transferring endoglycosidases are not. There is no evidence for the truth of this idea, but it is in line with Koshland's overall hypothesis[3], which is independent of his mechanisms for glycosidases, that enzymes transfer only those portions of the molecule for which they are specific.

3.4 *Fission and synthesis of chain glycosidic linkages*

The kinetics of the action of β-amylase can not be easily explained on the assumption of random association between substrate and enzyme followed by disjunction into enzyme and products. Bailey and French[82] have shown by statistical methods that about four glycosidic bonds per encounter are broken (to give maltose) by sweet potato β-amylase acting on amylose. Completion of enzymic action does not lead to dissociation of the enzyme substrate complex and only reversal of the original association process gives back enzyme and free substrate. These authors also showed that the production of four maltose units per encounter could not be explained by multiple active centres in the enzyme breaking four bonds simultaneously. Their results imply that the spatial relationship between the active centre and amylose chain can be altered to bring a new glycosidic bond for fission in contact with the active site, without dissociation of he enzyme-substrate complex.

The reverse case occurs with dextransucrase[21]. Here the acceptor polyglucosyl chain seems not to be dissociated from the enzyme while a series of donor sucroses react with it. Since the bonds in the chain are not all identical, this implies freedom for movement within the complex. Short polyglucosyl chains are not at any stage observed as products of the enzyme reaction. The overwhelmingly greater affinity of β-amylase for amylose over short-chain molecules makes it difficult to alter this reaction by using competing substrates; however, a number of small sugar molecules have an affinity for dextransucrase of the same order as that of the dextran chains. They tend to block the acceptor site whenever the enzyme-dextran complex dissociates and short-chain products can now be detected.

The statements that "the enzyme-substrate complex does not break up after enzyme reaction is complete", and "the affinity between enzyme and substrate is so high that there is only a negligible chance of substrate escape between any one action of the enzyme and the next" cannot be differentiated experimentally. In other words, in the conventional representation of the Michaelis-Menten hypothesis,

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P},$$

the value of k_1 for the series of related long-chain substrates is so much greater than the other constants that it controls the observed mechanism of enzyme action. The celebrated "zipper" action of β-amylase thus reduces to a question of relative affinities.

3.5 *Affinity and specificity*

The reader will observe how many problems have been disposed of by appeal to the blanket concept of "affinity". It can not be disguised that this, in fact, is largely an appeal to ignor-

ance. Biological specificity, in spite of some successful pictorial interpretations of observed phenomena, still can not be explained in detail. It is known from observation that certain molecules bearing the glycosidic linkage will adhere specifically to the surface of a given glycosidase at the "active centre" and that, once there, a proportion of these molecules can undergo the necessary rearrangements to give the products of enzymic action. In the present state of knowledge it is highly speculative to say more. Nevertheless, the immense accumulation of knowledge about the specificity of some of the glycosidases makes it very difficult to stop at this point. A great deal is known about this specificity, but next to nothing about the primary, secondary and tertiary structure of the protein and its exact specificity as an enzyme. This, however, does not mean that attempts to explain specificities in terms of carbohydrate stereochemistry, of which that of Gottschalk[83] is perhaps the best known, are valueless, but rather that they can not be checked by comparison with the structure to which they are expected to apply. These theories depend essentially on observing which parts of the structure of a substrate are apparently involved in specific binding to the enzyme. By examining models of various conformations of the glycosidic substrates, it is possible to deduce the conformation in which all the known substrates have the same relation between the centres of specificity.

This is an intellectual exercise that has appealed to most workers in the field and indeed its performance is essential for any valid final theory of enzyme mechanism. For example, the accepted interpretation of the conformation of β-glucosides puts the aglycone into an equatorial position on the glycopyranose ring, but Blom[84] has proposed a conformation that puts it in an axial position. Now there exist two classes of β-glucosidases, those much hindered by *ortho*-substitution in the aglycone of aryl β-glucosides and those not so hindered. Construction of models shows that *ortho*-substituted aglycones are much hindered in their rotation about the glycosidic linkage in the Blom conformation but not in the Reeves conformation. It has been shown[38] that the impairment of the action of β-glucosidases by *ortho*-substitution is, in general, not due to a lowered affinity of the substrate for the enzyme but to a hindering of the decomposition of the enzyme-substrate complex. Hence it may be assumed that for β-glucosidases hindered by *ortho*-substitution, the glucoside is bound in the Blom conformation, for the others, in the Reeves conformation. The Blom conformation will prevent free rotation of the aglycone to a position where attack on the glycosidic linkage can take place. This may be an attractive speculation, but in the absence of any direct knowledge of the structure of the enzymes involved it must remain a speculation. The full requirements for a "scientific explanation" have yet to be met.

The same remarks apply to various demonstrations that the specificity of the enzyme can be modified by modifying the enzyme itself. Murakami[85] apparently modified the relative specificity of the β-glucosidase of *Aspergillus niger* toward various substrates by chemical treatments; acceptance of his results is critically dependent on demonstration that his enzyme is a single chemical species. The same can be said for the demonstration by Whitaker[86] that the relative specificity towards various cellooligosaccharides of the cellulase of *Myrothecium verrucaria* can be altered by partial heat denaturation. However, the demonstration by Neely[87] that partial heat denaturation of dextransucrase makes maltose as well as sucrose a substrate is less open to this objection, since an activity is gained rather than lost. Ikenaka[88] has examined the effect on various enzymic activities of taka-amylase A, a crystalline highly purified enzyme, of the introduction of one phenylazobenzoyl ($C_6H_5N{=}NC_6H_4CO$-) group per molecule. Amylase activity is lost, phenyl α-maltosidase activity is enhanced, activity against *p*-nitrophenyl α-maltoside and maltotetraose is little affected. Ikenaka believes that the bulky group combines with a group close to the active centre in such a way as to preclude the approach of the bulky amylose molecule but not of smaller molecules. Enhancement of phenylmaltosidase activity will then be due to specific interactions between the introduced aromatic group and that of the substrate, thus increasing affinity.

If all these instances are taken at their face value, it is apparent that peptide chains are unfolding, or amino acid side-chains being altered, without any present certainty about what is going on, or just how the new steric

situations enforce a new discrimination between substrates. Koshland, Ray and Erwin[89] believe that they have conferred a new proteolytic activity on phosphoglucomutase, by altering the arrangement of the amino acids which control specificity. They assume that there are only a very few types of active centre and that what they do is controlled by the type of substrate that the specificity requirements of the molecule as a whole allow to be presented to it. Similar conclusions have been reached by Smith[90]. Enzyme specificity then becomes the same problem as that of specific enzyme induction or antibody formation. The steric relations which determine specificity in the "substrate" seem to be much the same in all cases[91]. If this hypothesis is true, and all recent knowledge in enzymology and protein chemistry favours it, then specificity becomes all important and its resolution in concrete terms a vital

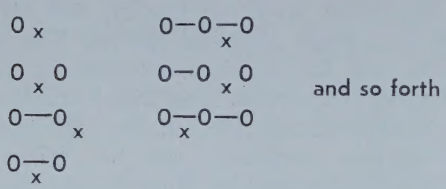


Figure 21

problem for the biochemist. Close cooperation between workers interested in protein structure and those interested in enzyme chemistry will be required. We may conjecture, for example, about the nature of the almost universal combination in glycopyranosidases of indifference to small changes in the groups bound to C2 and extreme sensitivity to changes on C3 in terms of conformational analysis. However, for its interpretation in terms of protein chemistry there is not a single piece of evidence, although our understanding of the nature of active centres in these terms is reasonably far advanced[90].

3.6 *Conclusions*

This review must end with the query with which it began—are glycosidases marked by differences in mechanism that separate them qualitatively from other enzymes, or are their peculiarities due only to the fact that the peculiar molecular environment which surrounds the bond to be broken must be fitted to a correspondingly specific portion of the enzyme? The essential organization that must be invisaged to permit a description of glycosidase action is a string of linearly disposed "specificity" centres lying in a defined relation to an active centre. The "specificity" centres could be occupied by the "tails" and "heads" of endoglycosidases, the "tail" of an exoglycosidase and its acceptor as in Figure 21. Thus enzymes exist (or could well exist) to fill all of them, but, until there is much more detailed knowledge of both the specificity and the activity centres, this scheme can only be regarded as a tidy system of classification.

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